CEREAL CHEMISTRY

CRITERIA OF THE VALIDITY OF ANALYTICAL METHODS USED BY CEREAL CHEMISTS

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(Read at the Convention, June, 1928)

An important undertaking of cereal chemists in recent years has been an attempt to determine the reliability of various methods of analysis, with a view to securing the standardization of technic that is a prerequisite for obtaining equivalent and comparable results in every laboratory. Tangible evidence of wide interest in this problem is afforded by the creation at the First International Conference on Flour and Bread Manufacture, held at Prague last year (Johnson, 1927), of a special international commission "to work out uniform analytical methods for cereal chemistry"; and by the publication this year of an official Book of Methods by the American Association of Cereal Chemists (1928).

The purpose of this paper is to comment briefly on the results of certain work of this nature carried out under the direction of the Committee on Methods of Analysis, of the American Association of Cereal Chemists, as set forth in its reports for 1924, 1925, and 1927. Our discussion will be limited to a consideration of biometric criteria available for testing the validity of conclusions concerning the differences between various methods of analysis that may be proposed, and that may be applied by a number of analysts. Such statistical methods are concerned with final results alone, and do not take cognizance of such things as cost of apparatus, expensiveness of materials used, or time involved, which may give decided practical advantages in favor of methods not rating so high in merit by purely statistical criteria, but of sufficiently close approximation to the results of the more accurate methods to justify their use.

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To us it appears that there are two fundamental criteria of the validity of any method that is tested on the basis of the analytical results obtained with a uniform sample of unknown content of the constituent being determined: (1) The average yield of the substance in question obtained by the different methods; (2) the consistency of the results obtained in reported determinations by the different technics.

With respect to the first of these, methods of analysis that give extremely high or extremely low yields may be suspected of being in error unless their validity is determined by such tests as the quantitative recovery of known additional amounts of the substance added to the original unknown. In comparing any two methods on the basis of criteria furnished by differences in average yield, the chemist must decide, on the basis of the fundamental theory underlying the analytical method employed, whether too high or too low yields are most probable. Thus in the present discussion the original unknown is in one instance the quantity of protein in flour. In this case, in the absence of other information as to the validity of the methods, it seems that those giving higher yields are more likely to be reliable than those giving lower yields, for failure to record nitrogen that is present is more likely than the recording of that not present. In the determination of ash content, a decision on *a priori* grounds cannot be so readily reached, as differences in yield may be too high because of incomplete oxidation of carbon on the one hand, or too low because of the loss of certain constituents by volatalization at high temperatures, on the other.

With reference to the second criterion, an important factor in the usefulness of any method is the consistency of the analytical results obtained when the method is used by *several* workers. If various analysts cannot obtain consis.ent results with a first method but can obtain closely agreeing results with a second, it seems that the second method is superior to the first (other advantages being reasonably similar).

We shall consider certain statistical criteria applicable to both of these problems.

A. Criteria of the Significance of Differences in Analytical Yields

The first problem is to determine whether the results of a series of analyses of the same sample of flour by different methods indicate significantly different yields by the several methods.

Three criteria may be applied. The one to be selected must be determined largely by the nature of the data available.

(a) Direct Comparison of the Differences of Mean Yields with Reference to Their Probable Errors

Workers are quite generally aware that when a moderately large series of analyses has been made on the same sample by two or more methods, their mean yields may be compared with due reference to their probable errors. The only point requiring emphasis here is that the formula given in textbooks is in many cases inadequate. Thus if N analysts make determinations by any two methods, A and B (where r_{AB} is the correlation between

$$E_{(\overline{A}-\overline{B})} = 0.67449 \sqrt{\frac{\sigma^2_A + \sigma^2_B - 2r_{AB}\,\sigma_A\,\sigma_B}{N}} \dots\dots\dots\dots\dots\dots (i)$$

results for the two methods as obtained by each analysis, and σ_A and σ_B are the standard deviations of the analytical results obtained by those methods), $E_{(\overline{A}-\overline{B})}$ is the probable error of the difference between the mean yields obtained by the two methods. If the analyses are made under such conditions that there is no significant correlation between the individual analyses of the two series, the third term of the numerator will vanish or may be neglected.

The correlation between analyses made by two methods by the same analysts may be of such magnitude as to influence materially the size of the probable error of the difference between the average yields obtained for the two methods.

As a preliminary to this point it is desirable to indicate the wider significance of such correlations.

Suppose N analysts each determine a constituent of a sample of flour by two methods, A and B. It is impossible that all obtain exactly the same results by either of the two methods because experimental error cannot be completely eliminated in any method. Such errors should, however, be random errors. That is, an analyst should not have a definite tendency to obtain results that are generally too high or too low. If systematic errors[2] or personal equation be wholly lacking, there should be no correlation between analytical results obtained by workers in using two different methods. If, however, systematic errors occur to any extent in excess of what might be expected from random sampling, the correlation coefficient should have a value that is significant in comparison with its probable error, the magnitude of r_{AB} measuring the extent of existence of personal or laboratory equation.

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[2] Such "systematic errors" may be inherent in the personal equation of the analyst or in the standardization of equipment, technic or reagents used in both Method A and Method B. For this reason we have designated such systematic errors as "personal equation" or "laboratory equation."

As in any acceptable method of analysis errors should be small in comparison with the amount of the constituent to be measured, it does not seem wise to group these analytical results into the wide-range classes of the ordinary correlation table for the determination of r, unless the number of analyses is sufficiently large to assure the worker that the mid-ordinates of these classes will really represent the value of the analyses assigned to the several classes. Instead, the actual values of the analyses should be used, without grouping, in determining the correlations. This can be most efficiently done by employing a method for the determination of r in terms of moments about zero as origin (Harris, 1910).

We now examine the values of the correlation between errors of measurement[3] as computed from actual cereal chemistry data.

The correlation between the ash contents[4] of flour as obtained by the two methods (A and B) for each of the five samples given in the report of 1927 were as follows:

No. of sample	Correlation and probable error $r_{AB} \pm E_r$	Ratio of r_{AB} to E_r
1	$+0.4759 \pm 0.0769$	6.19
2	$+ .5944 \pm .0643$	9.24
3	$+ .7474 \pm .0439$	17.06
4	$+ .4710 \pm .0774$	6.09
5	$+0.3965 \pm 0.0838$	4.73

The correlations between the protein content of flour as determined by two methods for the 47 analysts who employed both methods, as given in the report for 1927, are:

Nature of sample	Correlation and probable error $r_{AB} \pm E_r$	Ratio of r_{AB} to E_r
Protein in wheat	$+0.6348 \pm 0.0587$	10.81
Protein in flour	$+ .6428 \pm .0577$	11.14
Protein in bran	$+0.5223 \pm 0.0715$	7.30

These results are of great significance. The coefficients range from (approximately) 0.40 to 0.75 and are from 4.7 to 17.1 times as large as their probable errors. Thus they are unquestionably statistically significant[5] and show that these analysts have a very material personal

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[3] As all analyses by both methods are made by the N analysts on the same sample of flour, the correlation between analytical results obtained by using two methods is in reality the correlation between errors of measurement made by the analyst in using the two methods.

[4] See Appendix.

[5] In this paper we consider only values of the correlation coefficient that are 2.5 or more times as large as their probable errors to be significant, as with such a ratio there is approximately only one chance in eleven of obtaining a coefficient of the given magnitude from a random sample of the same size drawn from uncorrelated pairs of determinations. As the ratios increase in magnitude the probability of the significance of the coefficients increases very rapidly.

or laboratory equation that tends to render analyses made by two methods by the same worker erroneous in the same direction. Workers who report results that are below the general average for one method will, in general, report results that are too low by another method. Similarly, workers who report results that are above the general average by one method will tend to report results that are too high when determined by a different method.

If we can accept these data as typical, we must conclude that concordance of results obtained by two different methods by the same worker furnishes no final proof that he is really reporting the true value for his sample. Both results may be either too high or too low. The average values may in consequence be sensibly in error.

Turning now to the influence of these correlations on the probable errors of the differences in yields as determined by two methods, we may illustrate by the results in Table I for the two methods of determining ash content as given for 46 analysts included in the report for 1927. Further illustration is given in Table II, based on data drawn from the same source for protein determinations by 47 analysts.

TABLE I

MEAN ASH CONTENTS AS DETERMINED BY METHODS A AND B (1927 REPORT)

(Values expressed in per cent)

Sample No.	Mean yields* $\overline{A}$ and $\overline{B}$		Difference $(\overline{B}-\overline{A})$	$E_{(\overline{B}-\overline{A})}$	Uncorrected probable errors $\dfrac{(\overline{B}-\overline{A})}{E_{(\overline{B}-\overline{A})}}$	Corrected probable errors $E_{(\overline{B}-\overline{A})}$	$\dfrac{(\overline{B}-\overline{A})}{E_{(\overline{B}-\overline{A})}}$
	Method A	Method B					
1	0.4757 ± 0.0009	0.4783 ± 0.0009	$+0.0025$	0.0013	$+1.92$	0.0010	$+2.65$
2	$.6881\pm0.0009$	$.6877\pm0.0011$	$-\ .0004$	$.0014$	-0.28	$.0009$	-0.44
3	$.5514\pm0.0010$	$.5551\pm0.0012$	$+\ .0037$	$.0016$	$+2.37$	$.0008$	$+4.50$
4	$.5789\pm0.0008$	$.5833\pm0.0010$	$+\ .0044$	$.0013$	$+3.36$	$.0010$	$+4.56$
5	0.5534 ± 0.0007	0.5577 ± 0.0010	$+0.0043$	0.0012	$+3.42$	0.0010	$+4.33$

*Standard deviations given in Table V.

In these two tables the probable error is given as determined by both the conventional formula and that involving the correction due to the correlation. The influence of the correlation coefficient on the ratios to their probable errors of these differences in the means is very material. In the ash series, the ratios derived from the uncorrected probable errors range from -0.28 to $+3.36$, whereas the ratios obtained with the corrected probable errors range from -0.44 to $+4.56$. Thus it is clear that the differences are much more clearly significant when the full formula for the probable error is employed. In the case of the differences in the protein determinations shown in Table II, the numerical values of the ratios are also increased, but not to such an extent as to justify the differences in

the mean contents as determined by the two methods being considered statistically significant.

TABLE II

MEAN PROTEIN CONTENTS AS DETERMINED BY METHODS A^* AND B† (1927 REPORT)

(Values expressed in per cent)

Sample	Mean Yields‡ $\overline{A}$ and $\overline{B}$		Difference $(\overline{B}-\overline{A})$	Uncorrected probable errors		Corrected probable errors	
	Method A	Method B		$E_{(\overline{B}-\overline{A})}$	$\dfrac{(\overline{B}-\overline{A})}{E_{(\overline{B}-\overline{A})}}$	$E_{(\overline{B}-\overline{A})}$	$\dfrac{(\overline{B}-\overline{A})}{E_{(\overline{B}-\overline{A})}}$
Wheat	16.3083 ± 0.0186	16.2930 ± 0.0167	—0.0153	0.0250	—0.61	0.0152	—1.01
Flour	12.5443 ± 0.0100	12.5383 ± 0.0114	— .0060	.0151	— .39	.0091	—0.65
Bran	16.0798 ± 0.0205	16.0753 ± 0.0221	—0.0045	0.0302	—0.15	0.0209	—0.21

*Official A. A. C. C. method.
†Collaborators' own methods.
‡Standard deviations given in Table VI.

These results require a brief consideration from the chemical side.

It will be clear from the differences and the ratios of the differences to their corrected probable errors, in Table I, that in four cases out of five a significantly higher ash content is determined by the use of Method B (i.e., with added glycerol-alcohol) than by the use of Method A. In the protein determinations (Table II), Method B is in each case the collaborator's own method, and so the constants in this case really represent an average of several methods. It is, therefore, not comparable to Method A (the official A.A.C.C. method) in the same sense as in the ash determinations. It is interesting to note, however, the complete absence of significant differences in mean yield between the results of the official method and those obtained by the methods selected by the collaborating analysts. So far as this criterion is concerned, and so far as this series of analyses may be considered typical, the results indicate no superiority for the official method.

(b)   A Criterion of the Deviation of the Yield of a Given Method from the Mean Yield of a Series of Methods

In some cases a large number of analytical methods may be tried and the number of analyses by each method may be too small to make the comparison of the results of all pairs of methods practicable. Furthermore, the work of comparing all possible pairs of methods would be onerous, as the number of such comparisons would be $\frac{1}{2}n(n-1)$, where n is the number of methods. In the determinations of protein content by 27 different methods, as given in the 1924 report, 351 pairs of differences would have to be determined in order to make individual comparisons for all methods. Even with such a series of differences and their probable errors calculated, it would be difficult to select

methods that are significantly different in yield, unless the number of anaylses by each method was very large. When the number is not large, we may apply a method given by Pearson (1906) for determining the significance of the deviation of the mean of a subsample from the mean of a sample, with a view to ascertaining whether some of the methods may give results that may be reasonably considered significantly higher or lower than the average result obtained by all the methods tried. Such tests may at least indicate what particular methods should be more rigorously examined as to accuracy.

Let M be the mean of the sample, m, the mean of the sub-sample, Σ the standard deviation of the sample and σ the standard deviation of the sub-sample, based on N and n individuals respectively. Then the probable error of the deviation of the mean of the sub-sample (in this case the average result of analyses by any one technic) from the mean of all analyses is

$$0.67449\sqrt{\frac{\Sigma^2}{N} + \frac{\sigma^3}{n}\left(1 - 2\frac{n}{N}\right) - \frac{n(M-m)^2}{N(N-n)}} \quad \ldots\ldots\ldots\ldots\text{(ii)}$$

As an example we choose the work on protein determinations as given in the report of 1924. Therein results are given for 5, 6, or 7 analysts using the 27[6] methods, or variations of methods, of determining nitrogen.

Our findings for the application of Pearson's criterion to these data are given as the ninth column from the left (headed $\frac{m-M}{E_{(m-M)}}$) of Table III. We note that in only 3 cases out of 27 does this ratio exceed 2.5. Thus, so far as it can be shown by a series of analyses of this size, there is little differentiation between the yields given by the several methods.

A feature of some interest from the standpoint of methodology is the progressive change in the ratio as we pass from the shorter periods of digestion with their significantly lower yields, to the longer periods of digestion with their probably significantly higher yields.

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[6] It will be noted that in Table II of the report cited, Methods 3 and 6 in the list (numbering from the top) are identical, being repeated in the second case in a different series. For the purpose of this paper these two have been averaged for each collaborator and the result given as Method 3, Method 6 being dropped from the table.

TABLE III

COMPARISON OF MEAN YIELDS AND STANDARD DEVIATIONS OF PROTEIN CONTENT AS DETERMINED WITH DIFFERENT METHODS (1924 REPORT)

Method No.	Sodium sulphate	Red mercuric oxide	Sulphuric acid	Time of digestion	Sodium sulphide	No. of collaborators	m	$m-M$	$E_{(m-M)}$	$\dfrac{m-M}{E_{(m-M)}}$	σ	$\sigma-\bar{\sigma}$	$\dfrac{\sigma-\bar{\sigma}}{E_{\bar{\sigma}}}$
1	7.5	0.7	15	120	25	7	12.817	+0.0069	0.0455	+0.2	0.1625	—0.0014	—0.1
2	7.5	.7	20	120	25	7	12.856	+ .0455	.0417	+1.1	.1448	— .0192	—0.7
3	7.5	.7	25	120	25	7	12.860	+ .0498	.0471	+1.1	.1700	+ .0061	+0.2
4	7.5	.7	30	120	25	7	12.797	— .0130	.0520	—0.3	.1923	+ .0283	+1.0
5	5.0	.7	25	120	25	7	12.827	+ .0170	.0481	+0.4	.1744	+ .0104	+0.4
7	10.0	.7	25	120	25	7	12.833	+ .0228	.0421	+0.5	.1466	— .0174	—0.6
8	5.0*	.7	25	120	25	7	12.879	+ .0684	.0426	+1.6	.1490	— .0150	—0.5
9	7.5*	.7	25	120	25	7	12.870	+ .0598	.0493	+1.2	.1801	+ .0161	+0.5
10	10.0*	.7	25	120	25	7	12.857	+ .C470	.0595	+0.8	.2258	+ .0618	+2.1
11	7.5	.5	25	120	20	7	12.789	— .0216	.0464	—0.5	.1670	+ .0030	+0.1
12	7.5	.7	25	120	25	7	12.880	+ .0698	.0508	+1.4	.1872	+ .0232	+0.8
13	7.5	.9	25	120	30	7	12.817	+ .0070	.0457	+0.2	.1633	— .0007	—0.0
14	7.5	.5†	25	120	20	5	12.822	+ .0118	.0371	+0.3	.1017	— .0623	—1.8
15	7.5	.7†	25	120	25	5	12.782	— .0282	.0344	—0.8	.0902	— .0738	—2.1
16	7.5	.9†	25	120	30	5	12.812	+ .0018	.0330	+0.1	.0838	— .0802	—2.3
17	7.5	.1‡	25	120		7	12.826	+ .0155	.0439	+0.4	.1549	— .0091	—0.3
18	7.5	.25‡	25	120		7	12.817	+ .0070	.0557	+0.1	.2088	+ .0449	+1.5
19	7.5	.5‡	25	120		7	12.746	— .0645	.0558	—1.2	.2092	+ .0452	+1.5
20	7.5	.7	25	30	25	7	12.487	— .3230	.0730	—4.4	.2845	+ .1205	+4.1
21	7.5	.7	25	45	25	7	12.667	— .1430	.0404	—3.5	.1382	— .0258	—0.9
22	7.5	.7	25	60	25	7	12.714	— .0959	.0483	—2.0	.1756	+ .0116	+0.4
23	7.5	.7	25	75	25	7	12.841	+ .0312	.0451	+0.7	.1609	— .0031	—0.1
24	7.5	.7	25	90	25	7	12.854	+ .0441	.0490	+0.9	.1788	+ .0148	+0.5
25	7.5	.7	25	105	25	7	12.914	+ .1041	.0356	+2.9	.1143	— .0496	—1.7
26§	7.5	.7	25	120	25‖	6	12.775	— .0352	.0526	—0.7	.1796	+ .0156	+0.5
27	7.5	.7	25	120	25‖	6	12.817	+ .0065	.0486	+0.1	.1627	— .0013	—0.0
28§	7.5	0.7	25	120	25	7	12.903	+0.0927	0.0370	+2.5	0.1213	—0.0427	—1.4

*Potassium sulphate.
†Yellow mercuric oxide.
‡Copper sulphate.
§Pumice used instead of zinc for bumping.
‖Potassium thiosulphate.

$E_{\bar{\sigma}}$ (for n =7) = ±0.0296
(for n =6) = ±0.0319
(for n =5) = ±0.0350

Further work on this particular phase of the problem was undertaken by the committee for 1924-25. The data from its report have been studied by the same method. Here, however, we have used the more approximate form of the expression (for greater ease of calculation), which we found adequate for this series of data. Thus

$$E_{(m-M)} = 0.67449 \sqrt{\frac{\sigma^2}{n}} \quad \dots\dots\dots\dots\dots \text{(iii)}$$

This is the product of χ_1 Miss Gibson's tables (1926) and the standard deviation of the sub-sample.

The results are given as Table IV. On the first computation, the shorter periods of digestion gave significantly lower yields and the longer gave higher yields than the general average. Obviously these lower yields represent incomplete digestion and their inclusion has reduced the mean yield of the whole sample. We have therefore recalculated the criterion, progressively eliminating the lower digestions from the sample until there was no significant difference in the yields of the remaining digestions. Thus we find for wheat samples Nos. 1 and 3 no significant difference in the mean yield for periods of digestion of 45 minutes or over, and for wheat sample No. 2 no significant differences for periods of 60 minutes and over.

TABLE IV

DEVIATIONS OF MEAN PROTEIN DETERMINATIONS FOR INDIVIDUAL DIGESTIONS FROM GENERAL MEAN
(Values expressed in per cent)

Period of digestion	Mean yield	Standard deviation of sub-sample	Original sample			Final sample		
			$m-M$	$E(m-M)$	$\dfrac{m-M}{E(m-M)}$	$m-M$	$E(m-M)$	$\dfrac{m-M}{E(m-M)}$
Wheat Sample No. 1								
15 min.	12.1064	0.4649	—0.5416	0.0946	—5.7	....	....	....
30 min.	12.5518	.1561	— .0961	.0317	—3.0	....	....	....
45 min.	12.6800	.1701	+ .0320	.0346	+0.9	—0.0742	0.0346	—2.2
1 hr.	12.7236	.1598	+ .0757	.0325	+2.3	— .0306	.0325	—0.9
1 hr. 15 min.	12.7900	.2088	+ .1420	.0425	+3.3	+ .0358	.0425	+0.8
1 hr. 30 min.	12.7673	.1846	+ .1193	.0375	+3.8	+ .0130	.0375	+0.4
1 hr. 45 min.	12.7818	.1571	+ .1339	.0320	+4.2	+ .0276	.0320	+0.9
2 hr.	12.7827	.1580	+ .1348	.0321	+4.2	+ .0285	.0321	+0.9
Wheat Sample No. 2								
15 min.	10.5027	.2812	— .4169	.0572	—7.3	....	....	....
30 min.	10.8755	.0968	— .0442	.0197	—2.2	....	....	....
45 min.	10.9264	.1188	+ .0067	.0242	+0.3	....	....	....
1 hr.	10.9609	.1010	+ .0413	.0205	+2.0	— .0496	.0205	—2.4
1 hr. 15 min.	11.0100	.1258	+ .0903	.0256	+3.5	— .0005	.0256	—0.0
1 hr. 30 min.	11.0282	.1340	+ .1085	.0273	+4.0	+ .0176	.0273	+0.6
1 hr. 45 min.	11.0345	.1294	+ .1149	.0263	+4.4	+ .0240	.0263	+0.9
2 hr.	11.0191	.1497	+ .0994	.0304	+3.3	+ .0085	.0304	+0.3
Wheat Sample No. 3								
15 min.	13.1664	.4601	— .5632	.0936	—6.0	....	....	....
30 min.	13.6427	.1252	— .0868	.0255	—3.4	....	....	....
45 min.	13.7718	.1566	+ .0423	.0319	+1.3	— .0661	.0319	—2.1
1 hr.	13.8200	.1169	+ .0905	.0238	+3.8	— .0179	.0238	—0.8
1 hr. 15 min.	13.8627	.1812	+ .1332	.0368	+3.6	+ .0248	.0368	+0.7
1 hr. 30 min.	13.8700	.1764	+ .1405	.0359	+3.9	+ .0321	.0359	+0.9
1 hr. 45 min.	13.8373	.1456	+ .1077	.0296	+3.6	— .0006	.0296	—0.0
2 hr.	13.8655	0.1247	+0.1359	0.0254	+5.4	+0.0276	0.0254	+1.1

These computations do not assume to allow for any differences in the heat-producing capacity of the digestion burners used by the collaborators. Details concerning this factor are not given in the report.

That it is of importance in standardizing digestion time has been shown by Coleman, Fellows, and Dixon (1925).

In view of the results here obtained, it seems desirable that further extensive studies of protein determinations be made with careful standardization of heat sources. Recommendations regarding this have been made by the committee for 1925-26, but we know of no adequate studies of the length of periods required.

(c) Application of the Method of Intra-Class Correlation

Under more extreme conditions of large numbers of methods and small numbers of determinations by each method, we may test for the existence of differentiation in the yields obtained by the several methods by determining the intra-class correlation between the results of the analyses by the same methods as applied by different analysts. Thus we may refer again to the protein determinations by 27 different methods (see Table III), as given in the 1924 report.

While analysts vary in the skill with which they apply any method, it should be clear that if some methods tend to give abnormally high while others tend to give abnormally low results, there should be a correlation between the results obtained by different analysts in applying one and the same method. In Table III the methods are given in the horizontal arrays and the results of the various analysts form the vertical columns. We desire to measure any differentiation in the arrays. To determine this correlation we merely make every possible permutation of the results of the workers applying a given method, and combine the results in the form of a correlation table for all the methods employed. Such a table is the symmetrical correlation surface. In practice, the correlations are determined from moments without the formation of tables (Harris, 1913). In our illustration the classes are defined by the methods employed. The variates within the classes are the analytical results obtained by the n different analysts. Working from the formula:

$$r_{p_1 p_2} = \frac{\dfrac{S\{[\Sigma(p)]^2\} - S(p^2)}{S[n(n-1)]} - \left\{\dfrac{S[(n-1)\Sigma(p)]}{S[n(n-1)]}\right\}^2}{\dfrac{S[(n-1)\Sigma(p^2)]}{S[n(n-1)]} - \left\{\dfrac{S[(n-1)\Sigma(p)]}{S[n(n-1)]}\right\}^2} \quad \ldots\ldots (iv)$$

where p denotes protein content, Σ denotes summation within the individual classes (the results obtained by several workers employing the same methods), S denotes summation of classes, or of analyses through-

out the whole series as may be denoted by the context, $r_{p_1 p_2}$ is to be read as the correlation between the results of a first and a second worker employing the same method.

Applying this method to the data of Table II of the report already cited (in which we used the corrected columns $2a$ and $7a$ instead of 2 and 7 for reasons made clear in the report), we find an intraclass correlation of $r_{p_1 p_2} = +0.0620 \pm 0.0499$. The correlation between the results obtained by different workers employing the same methods, while positive, is very low indeed. It is only 1.24 times as large as its probable error. Its significance is, therefore, open to question.

Thus it is clear that, taking the series of analyses as a whole, there is no conclusive evidence that certain methods give significantly higher or significantly lower yields than the others. This result confirms the conclusions drawn under (b), above.

This is a mass result for the whole series of determinations. It does not preclude the possibility that one or very few of the 27 methods employed might in more extensive tests give significantly higher or lower yields than the others. It does indicate clearly that all these methods give reasonably concordant results so far as the total yield of protein is concerned. On the basis of this method of analysis, it cannot be definitely certain that any of these methods differ significantly from the others.

B. Criteria of Differences in the Consistency of Analytical Results Obtained by Different Methods

The consistency of the analytical results due to a given method may be measured in terms of the root-mean-square deviation of the individual analytical results from the mean of the whole series of analyses by the given method as applied to fractions of the same sample.

If two or more methods have each been employed to give analytical results for the same sample, their consistency may be directly compared in terms of their standard deviations, provided that the number of analyses by each method is sufficiently large that the standard deviations will have reasonably stable values.

Practically, two methods of procedure are available.

(a) Direct Comparison of Standard Deviations and Co-efficients of Variation

When the number of analyses made by two or more methods is sufficiently large, the variabilities by the two methods may be directly compared.

We may measure the variability in absolute units by the standard deviation, or in relative terms by the coefficient of variation. The former, σ, is given in terms of moments about zero as origin (Harris, 1910) by the equation

$$\sigma = \sqrt{\frac{\Sigma(x^2)}{N} - \bar{x}^2} \dots\dots\dots\dots\dots\dots\dots (v)$$

where x is the variable and $\bar{x}$ its mean value. When this standard deviation is related to the mean value of the variable, we have the variability in the universally comparable terms of the coefficient of variaton, V,

$$V = \frac{100\,\sigma}{\bar{x}} \dots\dots\dots\dots\dots\dots\dots\dots (vi)$$

We apply these criteria to the ash and protein determinations given in the report for 1927, and quote our results in Tables V and VI. The difference between two methods of making the same analysis may be compared with its probable error by the conventional formula for the probable error of the difference.

$$E_{(\sigma_B - \sigma_A)} = \sqrt{E_{\sigma_B}^2 + E_{\sigma_A}^2} \dots\dots\dots\dots\dots (vii)$$

The following points of interest to the student of analytical methods are to be noted.

(i) In the determinations of ash content there is an apparent tendency for Method B (involving the use of glycerol-alcohol) to give slightly more variable results than Method A (direct muffle method). In four of the five comparisons the standard deviations for Method B are greater than those for Method A. In three of the comparisons the ratios of the differences between the standard deviations to their probable errors are 2.26, 2.70, and 2.72. While such ratios furnish no final proof of the existence of a general difference in the variability of results by the two methods, they do furnish a strong indication that Method B gives less consistent (more variable) results than Method A.

The coefficients of variation and their ratios to their probable errors are also given, since they are somewhat more strictly comparable

TABLE V

STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF ASH CONTENTS AS DETERMINED BY METHODS A AND B (1927 REPORT)

(Values expressed in per cent)

Sample No.	Standard deviations* ×10⁶ σ_A and σ_B		Difference $\sigma_B - \sigma_A$ ×10⁶	$E(\sigma_B - \sigma_A)$ ×10⁶	$\dfrac{\sigma_B - \sigma_A}{E(\sigma_B - \sigma_A)}$	Coefficients of variation V_A and V_B		Difference $V_B - V_A$	$E(V_B - V_A)$	$\dfrac{V_B - V_A}{E(V_B - V_A)}$
	Method A	Method B				Method A	Method B			
1	9536 ±643	9155 ±637	− 381	905	−0.42	2.0048 ±0.1353	1.9144 ±0.1332	−0.0904	0.1899	−0.48
2	9987 ±674	11327 ±788	+1340	1037	+1.29	1.4520 ± .0980	1.6473 ± .1146	+ .1953	.1508	+1.30
3	9855 ±665	12302 ±856	+2447	1084	+2.26	1.7882 ± .1207	2.2165 ± .1543	+ .4283	.1959	+2.19
4	7843 ±529	10245 ±713	+2402	888	+2.70	1.3552 ± .0914	1.7566 ± .1222	+ .4015	.1527	+2.63
5	7743 ±522	10128 ±705	+2385	877	+2.72	1.4006 ±0.0945	1.8166 ±0.1264	+0.4159	0.1578	+2.64

*Mean yields given in Table I.

TABLE VI

STANDARD DEVIATION AND COEFFICIENTS OF VARIATION OF PROTEIN CONTENTS AS DETERMINED BY METHODS A* AND B† (1927 REPORT)

(Values expressed in per cent)

Sample	Standard deviations‡ σ_A and σ_B		Difference $\sigma_B - \sigma_A$	$E(\sigma_B - \sigma_A)$	$\dfrac{\sigma_B - \sigma_A}{E(\sigma_B - \sigma_A)}$	Coefficients of variation V_A and V_B		Difference $V_B - V_A$	$E(V_B - V_A)$	$\dfrac{V_B - V_A}{E(V_B - V_A)}$
	Method A	Method B				Method A	Method B			
Wheat	0.1870 ±0.0117	0.1671 ±0.0114	−0.0199	0.0163	−1.22	1.1476 ±0.0719	1.0259 ±0.0699	−0.1217	0.1003	−1.21
Flour	.1071 ± .0067	.1179 ± .0080	+ .0107	.0105	+1.03	0.8548 ± .0535	0.9405 ± .0641	+ .0857	.0835	+1.02
Bran	0.1974 ±0.0124	0.2208 ±0.0150	+0.0234	0.0195	+1.20	1.2284 ±0.0769	1.3735 ±0.0936	+0.1451	0.1212	+1.20

*Official A. A. C. C. method.
†Collaborators' own methods.
‡Mean yields given in Table II.

as they take into account the differences in mean yield by the two methods. These latter differences are, however, only small, and the ratios as a consequence are essentially similar to those for the standard deviations. Like the standard deviations, they furnish some evidence for the greater variability of results secured by Method B.

(ii) The official A.A.C.C. method of determining protein gives results that are just as variable as the heterogeneous series afforded by the collaborator's individual methods. This is striking, as it leaves the possibility that the official method is fully as variable in results as the worst of the collaborator's methods.

At this point attention may be invited to the relative variabilities of the three main chemical determinations upon wheat and flour, namely, protein, moisture, and ash. For this purpose we include here the coefficients of variation of the moisture determinations on wheat, flour, and bran as given for 51 analysts in the 1927 report.

Moisture in wheat .... $V = 1.452 \pm 0.097$
Moisture in flour .... $V = 1.454 \pm 0.097$
Moisture in bran .... $V = 2.359 \pm 0.158$

When these figures are compared with those in Tables V and VI, it will be noted that protein determinations show the least variability, about 1% on the average, while moisture determinations on wheat and flour come next at approximately 1.5%. Ash in flour shows somewhat higher variability, averaging about 1.7%. Protein and moisture determinations upon bran are clearly more variable than those on wheat and flour, especially in moisture determinations, where it rises to 2.4%.

For ash in flour it may be noted that the differences in variability for Methods A and B vary approximately from 0.1% to 0.4% for the five flours used. Furthermore, the coefficient of variation apparently shifts in its level as we pass from sample to sample. Thus, if the values obtained with samples of 47 and 50 may be regarded as reasonably stable, it appears that the character of the flour may influence the variability of its ash content as determined by different analysts, and also effects the discrepancy in this determination as between Methods A and B.

(b) Comparison of the Variability of a Sub-Sample with that of the Sample from Which It is Drawn.

The probable error of the standard deviation

$$E_\sigma = 0.67449 \frac{\sigma}{\sqrt{2n}} \dots\dots\dots\dots\dots\dots\text{(viii)}$$

is derived on the assumption that σ is either known or based on a

sample sufficiently large that the standard deviation thus determined may be legitimately used in place of the true but unknown value.

We certainly cannot accept the standard deviation as based on the results obtained by 5 to 7 analyses as at all reliable as the real measure of variability of the analyses by any one method. Neither can we take σ, as defined in equation (ii) above, as our measure of variability for any one method of analysis, since the relation

$$N \ (\Sigma)^2 = S(n.\sigma^2) \ + \ S[n(m-M)^2] \dots \dots \dots \dots (ix)$$

shows at once that the squared standard deviation of the sample as a whole represents not merely the summed squared standard deviations of the sub-samples but also includes the squared deviation of the means of the sub-samples from that of the sample. The contributions made by $S[n(m-M)^2]$ will be material even if the deviations $(m-M)$ be non-significant. If the methods of analysis be really differentiated in their yields, this may be an important factor in determining the variability of the results of all analyses. This is shown clearly in the protein determinations from the 1924 report, which we choose as an illustration of the use of this criterion. Here the average standard deviation of the analyses carried out by individual methods $\overline{\sigma_z}$, is smaller than the standard deviation of all analyses. Thus

$$\Sigma_z = 0.1930$$
$$\sigma_z = 0.1640$$

Now let us take $\overline{\sigma_z}$ the mean standard deviation of individual samples, as the most probable standard deviation of the analyses due to any one method. Assuming also that it is based on a sufficient number of cases that its probable error is negligibly small, the ratios of the deviations of a series of standard deviations of the results for individual methods, each based on n analyses, to their probable errors,

$$= \frac{\sigma_z - \overline{\sigma_z}}{0.67449\overline{\sigma_z}/ \sqrt{2n}} \dots \dots \dots \dots \dots \dots (x)$$

should be so distributed, in cases in which the sub-samples are really random samples from the same population, that when the signs of the deviations are disregarded,

$$\frac{S \left[\dfrac{\sigma_z - \overline{\sigma_z}}{0.67449\overline{\sigma_z}/ \sqrt{2n}} \right]}{N} = 1 \dots \dots \dots \dots (xi)$$

In work on a limited series this will be only approximately true. If a material proportion of the sub-samples differ in variability by an amount greater than that which might be expected as a result of random sampling from the same population, this value should exceed unity, and to an increasing amount as these differences are more significant.

The values given by (x) appear in the final column of Table III. Only in one method (No. 20, where the period of digestion is 30 minutes) does the ratio exceed 2.5.

Summing these values and dividing by $N = 27$ to obtain (xi) we find 0.96, indicating that in their general tendency these methods are not significantly differentiated with regard to variability. Method 20 does not, therefore, differ sufficiently from the general average to have any weight in the mass result.

Summary

The importance of obtaining equivalent and comparable results from all cereal-chemical laboratories is properly receiving serious consideration among workers in this field. Several collaborative studies of rather wide scope have already been made under the direction of the Committee on Methods of Analysis, of the American Association of Cereal Chemists.

The value of these efforts may be very largely lost unless careful and accurate study is also made of the resultant data by the refined mathematical methods of the biometrician.

The two criteria of fundamental importance for determining the validity of analytical methods when these are based solely on samples of unknown composition seem to us to be:

(1) Average yields and differences of average yields of the same constituent of wheat or flour as determined by two or more methods.

(2) Consistency of the results obtainable by any method of determining a constituent when applied by various workers.

In both cases the significance of differences in the results due to various methods must be tested by comparison with their probable errors. Formulae bearing on these points have been submitted, and illustration of their application has been given by use of data from the reports of the Committee on Methods of Analysis.

While the primary purpose of this paper is the indication of biometric methods suitable for dealing with tests of the validity of analytical technics, the computations have brought out the following main points concerning data already assembled:

(a)   Protein determinations:

The determination of protein in the flour is shown to be the most refined analysis among the three (moisture, ash, and protein), so far as consistency of results is concerned.

Providing adequate digestion is given, no one method of determining protein, as given in the reports, has been shown to be significantly different from any other method in the consistency of results obtained.

In no case has it been demonstrated that significantly higher yields are obtained by digesting longer than 60 minutes. This does not necessarily mean that higher yields by the use of longer periods of digestion may not be demonstrated by more extensive studies with careful standardization of all factors.

(b)   Ash determinations:

The glycerol-alcohol method of determining ash in flour gives significantly higher average yields than the straight muffle method, and it also tends to give significantly more variable results.

Greater variability, with different analysts, is found in ash determinations upon flour than in protein or moisture analyses.

(c)   Systematic errors in analyses:

The analytical determinations given in the reports studied show that there are very definite "systematic errors" or personal or laboratory equation among analysts. The demonstration of the existence of this systematic error is important for two reasons: (1) It shows that the concordance of results obtained by the same worker, using two different methods of making the same determination, furnishes no real proof that he is really reporting the true value for his sample; (2) This "systematic error" must be taken into account in determining the significance of the difference in the average yields obtained by two methods, differences always having a lower probable error (and a higher probability of significance) when correction for this factor is made.

Literature Cited

American Association of Cereal Chemists
    1924   Report of committee on methods of analysis (L. H. Bailey, chairman). Cereal Chem. 1:183-202.
    1925   Report of committee on methods of analysis (C. E. Mangels, chairman). Cereal Chem. 2:235-251.
    1927   Report of committee on methods of analysis (D. A. Coleman, chairman). Cereal Chem. 4:311-326.
    1928   Methods for the analysis of cereals and cereal products (with reference tables). (Compiled by committee on methods of analysis.)   Lancaster Press, Lancaster, Pa.

Coleman, D. A., Fellows, H. C., and Dixon, H. B.
    1925 A study of methods for making protein tests on wheat. Cereal
          Chem. 2:132-164.
Gibson, Winifred
    1906 Tables for facilitating the computation of probable errors. Bio-
          metrika, 4:386-393.
Harris, J. Arthur
    1910 The arithmetic of the product moment method of calculating the
          coefficient of correlation. Am. Nat. 44:693-699.
    1913. On the calculation of intra-class and inter-class coefficients of
          correlation from class moments when the number of possible
          combinations is large. Biometrika, 9:446-472.
Johnson, A. H.
    1927 Report of the First International Conference on Flour and Bread
          Manufacture in Prague, Czeckoslovakia. Cereal Chem. 4:495-500.
Pearson, K.
    1906 Note on the significant or non-significant character of a sub-sample
          drawn from a sample. Biometrika. 5:181-183.

APPENDIX

Elimination of Certain Analyses from Computations

The fixing of limits for the acceptability of results of analytical determinations is questionable when such is not based upon a study of the distribution or frequency curve and a consideration of the odds against obtaining extreme results. In the 1927 report, certain results by individual analysts were discarded, while in other cases all the results by an analyst using one or more methods were dropped from the averages by the committee. This was apparently done by inspection of the general results. We do not wish to assert here whether the action taken was in any specific case right or wrong, but we have adopted the principle for this study that if an analyst's result on a particular sample for one method is not acceptable, in the lack of accurate information of the odds as above it is wiser, in the selection of a population to give true values of the mean and standard deviation, to omit all the results of that analyst for that particular method. In this study this also has the advantage of keeping the number of individuals the same for any one method, thus making constants for each sample more readily comparable.

Accordingly we have omitted the results of the following analysts from our calculations.

Table I. Moisture determinations

Briggs, C. H.; Carr, Frank; Foster, C. H. (Asterisk apparently erroneously omitted from published table); Murphy, C. M.; Swanson, C. O.

Table II.   Protein determinations

Tibbling, E., Collaborator's method (Protein in flour an apparent misprint).

Table III.   Ash determinations

Carr, Frank, Method *B* (wrongly asterisked for Method *A*); Glasgow, W. E., Method *B;* Hall, W. E., Methods *A* and *B;* Maney, Methods *A* and *B;* Rosse, M. C., Methods *A* and *B* (asterisk accidentally omitted in published table for Sample 4*A*); Wilhoit, A. D., Methods *A* and *B*.

A CRITICAL STUDY OF SOME METHODS USED IN FLOUR COLORIMETRY

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(Read at the Convention June, 1928)

The color of flour is of great importance in the milling industry. Reliable methods for determining the color of flour or at least for finding a numerical expression for color, are therefore of great interest to the cereal chemist.

Bailey (1925) gives the following factors which together determine the color of flour.

1.   The granulation of the flour.

The finer a flour is ground the whiter it appears. This is due to the physical phenomena of the difference in the refraction of the light in the granules and the air which surrounds them.

2.   The influence of enzymes in the flour.

The influence of enzymes shows itself when a dough made of the flour is left standing in contact with air.

3.   The presence of finely ground bran particles.

The reddish brown pigment in the bran particles contributes to the color of both flour and dough.

4.   The presence of dirt and foreign matter, such as soil, fragments of weed seeds, and smut spores.

5.   The carotinoid pigments.

These pigments, which are present in a great variety of substances of vegetable or animal origin, are responsible for the yellowness of flour. Monier-Williams (1912) concluded from his ex-

periments that the principal yellow pigment in wheat flour was carotin $(C_{40}H_{56})$.

The only way to determine the combined effect of these five factors on the color of a certain flour is to compare a sample with a sample of standard flour. This method, therefore, has all the defects of any method in which a standard must be chosen that is not a well defined chemical compound. Moreover, it is not possible to give a numerical expression for the result observed. The method to be used in this comparison is the old Pekar test.

It will not be necessary to describe here this most popular and most commonly used flour test. By comparing the colors of the two flour samples in the dry state, the wet state, and after drying at an elevated temperature, this test gives the skilled observer very useful information on both color and grade of the flour. It lacks scientific precision because too many uncontrollable factors influence the results. For the Pekar test it is necessary to have a standard sample of flour. This is one of the most serious objections to this method, as the standard flour whitens with age. This difficulty can be only partly overcome by changing the standard flour at short intervals. A further objection is the personal observation error entering in when reading the color value. No two persons have just the same eye for shades of color.

William Jago (1911) used the Lovibond tintometer with Lovibond colorimeter glasses and tried to express the color of flour in definite numbers of yellow and red. He disposed of the necessary flour standard by using a standard white surface made of plaster of paris. This method was never applied extensively because different operators obtained different results. (See Kent-Jones [1927] and Coleman and Christie [1926]). The results obtained by Priest (1928), in the Bureau of Standards, in testing color sensitivity of different persons warrant further investigations on the applicability of the Lovibond tintometer in flour colorimetry. It must be understood that measuring the color of a flour in definite numbers of yellow and red can never give a complete record of the color of the flour. What is measured here is probably nothing but the amount of yellow and red pigments present.

In using the ash content of flour as a basis of color valuation a similar mistake is made. Ash content is used as a measurement for the grade of flour, but altho grade is an important factor, it does not necessarily determine the entire color.

For all practical purposes we may now say that the color of flour is really determined by two factors:

1. Carotinoid pigments.

2. Bran particles. The amount of these particles is closely related to the grade of flour.

As described above, a considerable part of the color of flour is due to carotinoid pigments, which give the flour its yellowness. Carotin is soluble in gasoline, and can easily be extracted from the flour by this solvent. By comparing the color of the gasoline extract of the flour with standard color solutions, the carotin content of the flour can be measured. This is the official method of the American Association of Cereal Chemists (1928). An objection to this method is that it takes a long time, which is a serious handicap for use in the mill. This is, however, very efficiently corrected by Coleman and Christie (1926). They make it possible to finish the test within two hours. The results obtained are the same as with the official method. A weak point of the official method is the standard solution. Not all the carotin extracts of different flours have exactly the same shade of yellow, and it is, therefore, rather difficult to find a standard solution with which to compare them. The fact that nearly every new worker in this field has proposed a new standard solution illustrates plainly the difficulty experienced with these color comparisons.

The best and most accurate solution of this problem would no doubt be the use of standard solutions made of pure carotin, as suggested by Palmer (1922). The objection to this is that pure carotin is hard to prepare and is too expensive for ordinary laboratory use. Carotin solutions are not stable in contact with the air. Therefore fresh standards would have to be prepared regularly.

Willstätter and Stoll (1913), in their original studies on carotin, used a solution of 0.25% alizarin in chloroform or a solution of 0.2% potassium chromate in water as color standards.

Winton (1911) was the first to make use of a 0.005% potassium chromate solution as a standard. This standard has been adopted by the A. A. C. C. The tint of this standard solution is sufficiently similar to that of the gasoline extract of most flours to make its use practical. There always remain, however, slight differences in shade. Holger Jørgensen (1927) has called attention to the fact that a dilute potassium chromate solution has an *undefined* color because the H-ion concentration of the solution has an influ-

ence on the shade of the color. Our experience agrees with this statement. We think, therefore, that the use of a well defined buffer solution for the preparation of the standard would be an improvement on the official standard.

Sprague (1928), describes a method of colorimetric measurement of chloroplast pigments, in which he uses as standard solutions, solutions made up with organic dyes, respectively napthol yellow, or orange G, and malachite green. We have made only a limited number of tests with these standard solutions, but our impression of them is very favorable.

Kent-Jones and Herd (1927), describe a new method which enables them to find a numerical expression for the color of flour. They, for the first time, not only determine color due to carotinoid pigments but also color due to bran particles. Therefore their method certainly merits attention. In the experimenal part of this paper we discuss this method in detail.

The principal point of their method is that they arrange for two independent extractions of the flour: one with gasoline, which deals with the yellow pigments of the flour; and the other with alkaline methyl alcohol which extracts the pigments from the branny matter. They state that neither solvent has any effect on the other pigment. The method consists of:

1. The two independent extractions.
2. By matching the depths of the colored extracts with standards made by the addition of measured quantities of standard color solutions to pure water.

The comparison is carried out in a specially designed colorimeter which, they claim, is more accurate than other types. Another advantage of this instrument is that it works with artificial light, and therefore makes the worker independent of daylight. As a color standard, Kent-Jones and Herd use a solution made up from potassium chromate and cobalt nitrate solutions. The authors claim that the extraction with methyl alcohol gives a good measure for the amount of finely ground bran in the flour, and therefore of the grade of flour. They claim that the methyl alcohol value shows a close correlation with the ash content of the flour, and that this figure is not affected when the flour is bleached.

Kent-Jones makes the gasoline extract according to the method of the A. O. A. C. This method is essentially the same as the official method of the A. A. C. C.

Twenty grams of flour is weighed into a wide-mouthed, glass-stoppered, 8-ounce bottle, and 100 cc. standard grade gasoline is added (we used light gravity gasoline furnished by the Standard Oil Company). The bottle is stoppered tightly, and the contents are thoroly mixed and shaken periodically for about 15 minutes. It is then allowed to stand for 16 hours (over night is convenient). At the end of this period the contents are reshaken and after standing a few minutes to allow the majority of the flour to settle, the moderately clear gasoline extract is filtered into a dry conical flask, through a No. 5 Whatman filter paper. The funnel is kept covered by a watch glass during the filtration, in order to prevent evaporation, and it is usually found necessary to refilter the first few cc. of the filtrate. When sufficient filtrate has been collected, 50 cc. is transferred to the Nessler glass, which is then inserted into the colorimeter.

As this method takes a long time, we used the technic given by Coleman and Christie (1926). However, instead of stirring the mixture of gasoline and flour with a "soda fountain" stirrer as they described, we closed the stoppered bottle tightly with a strong rubber band. The bottle was then placed in a shaking machine, which held four bottles at a time. The bottles were shaken vigorously for an hour, which gives, as Table I shows, exactly the same results as standing over night. Even half an hour shaking is sufficient, but to be absolutely sure, an hour was always taken.

TABLE I

INFLUENCE OF SHAKING TIME ON GASOLINE VALUE

Sample No.	Gasoline value		
	Shaken 15 min. stood over night	Shaken 60 min. and filtered	Shaken 30 min. and filtered
AI	1.49	1.43	1.48
AII	0.81	0.81	0.81
WRI	0.49	0.45	0.47

The standard color solution used was the one given by Kent-Jones and is made up as follows:

Ten cc. of a 0.5% potassium chromate solution and 1.5 cc. of a 10% solution of anhydrous cobalt nitrate are made up to 100 cc. with distilled water.

The original Kent-Jones colorimeter, which we used in our experiments, contains two Nessler tubes holding 50 cc. each. The Nessler tubes are lighted at the bottom by indirect artificial light, reflected by a dull white reflecting plate. On top of the Nessler tubes is a prism box, which allows the observation of the two tubes in a single field. A daylight filter is placed between the

prisms and the eye-piece. To make a determination, 50 cc. of the extract is poured into one of the Nessler tubes. The other tube is filled with about 40 cc. of distilled water, and a trial number of cubic centimeters of the particular standard solution is run from a burette into the latter tube. This is done until the color in both tubes matches closely. The number of cubic centimeters used gives the color value of the flour.

We thought it advisable to compare Kent-Jones' colorimeter with a regular Duboscq colorimeter. We used a 50 mm. plunger-type Spencer instrument. Readings of the same solutions in both instruments would give an idea of their comparative accuracy. The standard solution given by Kent-Jones is too concentrated for use in a Duboscq colorimeter. It was therefore necessary to dilute 10 cc. of this standard solution with distilled water to 100 cc. Kent-Jones' method expresses the amount of pigment present in 50 cc. of extract, in cubic centimeters of concentrated standard solution. With the Duboscq colorimeter, we find the amount of coloring matter present in 1 cc. of the extract, expressed in cubic centimeters of the dilute standard solution. The standard solution used in the Duboscq colorimeter had only 1/10 of the strength of the con-centrated standard solution. It is apparent that the figures found with Kent-Jones' method must be five times as high as those given by the Duboscq colorimeter. To obtain the same results, it is therefore necessary to divide by five the figures given by the Kent-Jones' method.

Gasoline extracts were made of several commercial flour sam-ples and the gasoline figure of each extract was determined with both colorimeters. The results of eight of these tests can be found in Table II. The gasoline value is given as the number of cubic centimeters of dilute standard, equivalent to the amount of color in 1 cc. of the extract.

TABLE II

COMPARISON OF KENT-JONES COLORIMETER AND DUBOSCQ COLORIMETER

Sample	cc. used by Kent-Jones method	Gasoline value, Kent-Jones	Gasoline value, Duboscq
1	14.8	2.96	1.49
2	14.9	2.98	1.43
3	14.9	2.98	1.48
4	5.7	1.14	0.80
5	5.6	1.12	0.81
6	5.4	1.08	0.81
7	4.7	0.94	0.69
8	2.2	0.44	0.42

Results show that with flour of high gasoline value (un-bleached flour), there was a big difference between the values ob-

tained with the different colorimeters. For instance, sample 1 gave, according to Kent-Jones' method, a gasoline value of 2.96; and in the Duboscq colorimeter, a value of only 1.49—a difference of 100%. The same flour after bleaching (sample 5) gave respectively 1.12 and 0.81—a difference of 38%. Sample 8, highly bleached flour, gave exactly the same results with both methods. We see from these tests that the differences between results are small with the low values, but are larger with the higher values. To determine which colorimeter was at fault, the following tests were made.

A large amount of gasoline extract was made from an unbleached flour. Of this extract, a series of dilutions was made as follows:

Ten cc. of the extract was pipetted into a volumetric flask of 100 cc. and filled up to the mark with clear gasoline. The same thing was done with 25, 50, and 75 cc. In this way five solutions were obtained, in which the amount of coloring matter was in the ratio of 10:25:50:75:100. It could, therefore, be expected that the gasoline values would show the same ratio, and that the gasoline value of the original liquid could be calculated from the others by multiplying by 10, 4, 2, and 4/3. The gasoline values of these extracts were made with both colorimeters. The results are given in Table III.

TABLE III

COMPARISON OF KENT-JONES COLORIMETER AND DUBOSCQ COLORIMETER
(Inorganic standard solution used.)

	Kent-Jones colorimeter			Duboscq colorimeter	
	cc. used	Gas. value	Gas. value, original extract	Gas. value	Gas. value, original extract
Original extract, cc.	11.9	2.38	2.38	1.31	1.31
{ 75 or. extract 25 gas.	8.2	1.64	2.20	0.99	1.32
{ 50 or. extract 50 gas.	4.8	0.96	1.92	0.65	1.30
{ 25 or. extract 75 gas.	2.0	0.40	1.60	0.32	1.28
{ 10 or. extract 90 gas.	0.7	0.14	1.40	0.13	1.30

The gasoline values made with the Duboscq colorimeter gave exactly the same value (1.30) for the original extract. The gasoline value of the original extract found with Kent-Jones' colorimeter differs from 2.38 to 1.40. This shows that the method used in the Kent-Jones' colorimeter is at fault.

Is it possible that the error is in the color standard? The standard solutions used in these tests were the standards given by

Kent-Jones. The same gasoline extracts used in Table III were now compared in the Kent-Jones' colorimeter with a standard solution as used by Sprague (1928). This standard is made by adding 3.4 cc. of 0.5% aqueous solution of naphthol yellow and 0.5 cc. of 0.5% aqueous solution of orange G. crystals to one liter of distilled water. The tint of this standard is identical with that of pure carotin dissolved in gasoline. The standard is equivalent to a carotin solution containing 1.89 mgm. of carotin per liter of solvent.

In the Duboscq colorimeter we used, as explained before, a solution with 1/10 of the strength of that used in the Kent-Jones colorimeter. The results of our tests are given in Table IV.

TABLE IV

COMPARISON OF KENT-JONES COLORIMETER AND DUBOSCQ COLORIMETER
(Organic dye standard)

	Kent-Jones colorimeter			Duboscq colorimeter	
	cc. used	Gas. value	Gas. value, original liquid	Gas. value	Gas. value original liquid
Original extract, cc.	7.3	1.46	1.46	1.34	1.34
75 or. extract 25 gas.	5.4	1.08	1.44	0.98	1.30
50 or. extract 50 gas.	3.5	0.70	1.40	0.68	1.36
25 or. extract 75 gas.	1.6	0.32	1.28	0.33	1.32
10 or. extract 90 gas.	0.7	0.14	1.40	0.13	1.30

We see that the gasoline value of the original extract is practically the same in all five cases, and for both colorimeters the small differences are within the limits of experimental error. This shows that the error in Kent-Jones' method is caused by the inorganic standard solution. Kent-Jones' method uses the standard solution in different concentrations. In some cases up to 15 cc. of the standard solution is diluted with 40 cc. of water, and in other cases only 2 cc. of the standard solution is diluted with the same amount of water.

Jørgensen (1927) has shown that the H-ion concentration of the chromate solutions has a substantial influence on their tint. He therefore dilutes his inorganic standard solution with strongly buffered solutions, thereby keeping the H-ion concentration and the tint of his solution constant. We applied the same principle in a new series of tests with the Kent-Jones colorimeter. The extracts were prepared in the same way, and the test was repeated with the Kent-Jones colorimeter, using both pure water and the buffered solutions. Sørensen's phosphate mixture, consisting of 95 parts primary potassium phosphate solution and 5 parts sec-

ondary sodium phosphate solution, both of 1/15 molar concentration, was used as a buffer solution. This liquid has a pH of 5.6. The results of these tests are given in Table V.

These tests show that better results can be obtained with the Kent-Jones method if care is taken that the pH of the standard solution remains the same.

TABLE V

COMPARISON OF GASOLINE VALUES OBTAINED WITH AND WITHOUT BUFFER SOLUTION

	Kent-Jones colorimeter				Duboscq colorimeter			
	Water		Buffer solution		Water		Buffer solution	
	Gas. value	Gas. value, original extract	Gas. value	Gas. value, original extract	Gas. value	Gas. value, original extract	Gas. value	Gas. value, original extract
Original extract, cc.	4.70	4.70	2.60	2.60	2.55	2.55	2.60	2.60
50 or. extract 50 gas.	2.20	4.40	1.40	2.80	1.21	2.42	1.30	2.60
75 or. extract 25 gas.	0.80	3.20	0.72	2.88	0.64	2.56	0.60	2.40

The methyl alcohol figure, according to Kent-Jones and Herd, indicates the grade of flour. The extracted pigment is the coloring material from the small bran particles in the flour.

Kent-Jones makes the methyl alcohol extract as follows: "Twenty grams of flour are introduced into a wide-mouthed glass-stoppered 8-ounce bottle, and 50 cc. of distilled water is added. The stopper is inserted and the bottle shaken to effect a complete mixing of the flour and water. Five cc. of a normal caustic soda solution is then added, and the bottle is again vigorously shaken. The bottle and its contents are allowed to stand for one hour with intermittent shaking, say at approximately every ten minutes. At the end of the hour, 100 cc. of methyl alcohol is added, the whole shaken thoroly for a few minutes, and then allowed to stand overnight (16 hours). In the morning the supernatant liquid is decanted off into a beaker and the glutenin is precipitated by the addition of N/5 hydrochloric acid. The glutenin is precipitated at a pH of 6.4. He uses the indicator bromthymol blue externally. This can be done with a spotting tile, or by taking out a few cc. in a small test tube and adding the indicator, the change being indicated by the formation of a light olive color. The precipitated glutenin is allowed to stand for one hour and is then centrifuged, the glutenin being thrown to the bottom as a compact disk. The supernatant liquid is filtered through a No. 5 Whatman filter paper into a clean, dry beaker. Fifty cc. of this filtrate is taken and one cc. of normal caustic soda is added; this is sufficient to render the

methyl alcohol mixture distinctly alkaline and the color of the pigment, which is destroyed in acid solution, is reproduced. The 51 cc. is then poured into the Nessler glass of the colorimeter."

We observed that during the precipitation of the glutenin the pH of 6.4 should be reached very accurately, otherwise it is impossible to get a clear liquid. A skilled worker, however, can do very well without the use of the indicator. The liquid itself acts as an indicator, and when the hydrochloric acid is added carefully drop by drop, the point at which the liquid turns nearly colorless and the glutenin starts to precipitate as a flaky mass, can be readily observed. Even a slight excess of hydrochloric acid will cause a milky white liquid which cannot be cleared by centrifuging.

The extract prepared according to the above described method is now compared with the standard solution. This standard is made up as follows:

Fifty cc. of 0.5% potassium chromate solution and 2.0 cc. of 10% anhydrous cobalt nitrate solution, are made up to 100 cc. with distilled water.

We made methyl alcohol extracts of several flour samples. The methyl alcohol value was determined in both the Kent-Jones and the Duboscq colorimeter. The standard solution used was the one given above. For use in the Duboscq colorimeter it was diluted ten times. The results are given in Table VI, as cc. of diluted standard solution, equivalent to one cc. of the extract.

TABLE VI

METHYL ALCOHOL VALUES OF DIFFERENT FLOURS

| Sample No. | Kent-Jones colorimeter | | Duboscq colorimeter |
	cc. used	Methyl alcohol value	Methyl alcohol value
1	12.5	2.50	2.56
2	11.2	2.24	2.32
3	11.0	2.20	2.21
4	9.1	1.82	2.03
5	6.5	1.30	1.17
6	6.9	1.38	1.31
7	8.25	1.64	1.66
8	7.7	1.54	1.63

In these tests the figures given by the two colorimeters agree very well. This agreement was not expected because of the disagreement in the gasoline values. This is probably due to the more diluted standard, and to the fact that the difference in the necessary amount of cc. was not so big. The amounts used differ only from 12.5 cc. to 6.5 cc.

With these extracts it was difficult to obtain a close match between the color of the extract and of the standard solution. The

standard solution showed too much red. With the Kent-Jones colorimeter a fairly good match could be obtained, but there was always a difference in the shade of yellow. With the Duboscq colorimeter, the difference was much more pronounced because in this thin layer the red of the standard solution shows up more.

To see if the pH has influence on the methyl alcohol value, the following test was made:

From a sample of flour, the methyl alcohol extract was made in the usual way: 25 cc. of this extract was diluted to 100 cc. with a mixture of two parts of pure methyl alcohol and one part distilled water. The same thing was done with 50 cc. and 75 cc. of this extract. Out of the readings obtained with these solutions, the value of the original liquid can be found by multiplying by 4 or 2, or 4/3. The readings were done with the Kent-Jonés colorimeter, using distilled water and a buffer solution of the same composition as used for the gasoline values. The results are given in Table VII.

TABLE VII
METHYL ALCOHOL VALUES WITH DISTILLED WATER AND WITH A PHOSPHATE BUFFER SOLUTION

	Distilled water		Buffer solution	
	Methyl alcohol value	Methyl alcohol value, original liquid	Methyl alcohol value	Methyl alcohol value, original liquid
Original extract, cc.	1.64	1.64	1.52	1.52
{ 75 original extract { 25 methyl alcohol	1.26	1.68	1.20	1.60
{ 50 original extract { 50 methyl alcohol	0.92	1.84	0.82	1.64
{ 75 methyl alcohol { 25 original extract	0.52	2.08	0.42	1.68

These figures show that the use of distilled water also gives a slight error, much smaller, however, than the one found with the gasoline values. This error is corrected by the use of the buffer solution. The results do not check up well, probably owing to errors caused by the observers' difficulty in getting an exact match of colors. With the Duboscq colorimeter it was impossible to get a match of the colors with this standard, and the results are therefore not recorded.

We found it necessary to look for another standard solution, which would give better satisfaction. As the standard solution made with potassium chromate and cobalt nitrate seemed to be too red, it was obvious to try potassium chromate alone.

A new standard solution containing 0.005% potassium chromate was prepared by diluting 10 cc. of a 0.5% solution of

potassium chromate to 100 cc., and 10 cc. of this solution was once more diluted to 100 cc. The last time, a buffer solution of of the same composition as given above was used to dilute the standard solution. This standard solution for methyl alcohol values is the same as that given for gasoline values by the official methods of the A. A. C. C. except for the use of the buffer solution. This proved to be a good standard solution for the methyl alcohol extracts of the samples of flour tested by us.

A series of tests was made to see if we could find the same correlation between the ash content and the methyl alcohol value of the flour as Kent-Jones. All these tests were made with the Duboscq colorimeter, which we prefer to the Kent-Jones colorimeter because it is easier to handle and gives just as accurate results. For these tests the potassium chromate standard solution described above was used.

Results are given in Table VIII. Samples A to WR2 are commercial flour samples. Samples HJ1, HJ2, and HJ3 are flours milled from the same wheat but of different extraction. HJ1 was a flour made from the first middlings, HJ2 a clear, and HJ3 a low-grade flour. Samples 0, ½, 1, 1½, are samples of the same unbleached flour, but to ½ was added ½% of very finely ground bran, to 1 was added 1%, and to 1½, 1½%. The bran was ground as fine as possible in a laboratory hand mill and was thoroly mixed with the flour by shaking the weighed amount of bran and flour together in a glass bottle.

We found that the methyl alcohol extraction of flour was not complete in 16 hours. Samples were tested according to the directions given by Kent-Jones. These extracts were left standing over night, and worked up in the morning *without* shaking. Another series of methyl alcohol extracts of the same flour samples was made and worked up after standing over night (16 hours) and after having been thoroly shaken in the morning. A third series of flour extractions was kept standing for 40 hours, and was shaken thoroly after the first 16 hours and at the end of 40 hours. The results are given in Table VIII. All tests were made in duplicate and the extracts were compared in a Duboscq colorimeter, on the same day, against the same standard solution. The standard solution used was 0.005% potassium chromate solution.

Results show that it is absolutely necessary that the extracts are shaken at the end of the 16 hours, as the results, without shaking, are only about 70% of those obtained after shaking. The

results show, furthermore, that the extraction is not complete after 16 hours. The figures obtained after 16 hours are only about 85% of those obtained after 40 hours.

The ash determinations given in Table VIII were made according to the official glycerol-alcohol method. In general, we get the same results as Kent-Jones. There is a correlation between ash content and methyl alcohol value.

TABLE VIII

INFLUENCE OF EXTRACTION TIME UPON THE METHYL ALCOHOL VALUE AND COMPARISON BETWEEN METHYL ALCOHOL VALUE AND ASH CONTENT

	Standing 16 hours	Standing 16 hours and shaken	Standing 40 hours and shaken	Ash content
A0, unbleached flour	1.19	1.53	1.77	0.40
A1, same flour bleached	1.00	1.52	1.68	0.42
B	1.09	1.60	....	0.43
C, soft winter wheat flour	1.35	1.70	2.32	0.43
D, soft winter wheat flour	1.11	1.77	....	0.44
E, mixture of hard and soft winter wheat	1.44	2.22	2.45	0.54
F1	1.55	2.15	2.55	0.49
F2	1.55	2.04	2.37	0.48
WR1	1.04	1.28	1.63	0.50
WR2	1.03	1.44	1.63	0.51
HJ1, flour from first middlings	0.66	1.08	1.36	0.35
HJ2, clear from same wheat	2.22	3.12	3.48	0.71
HJ3, low grade flour same wheat	7.10	....	8.50	1.43
0, unbleached flour	1.12	1.40	1.72	0.43
½, same flour +½% bran	1.30	1.68	2.00	0.46
1, same flour +1% bran	1.46	....	2.48	0.48
1½, same flour +1½% bran	1.61	2.10	2.68	0.51

To find out if the temperature at which the extraction was carried out had any influence on the final result, the following test was made. Of a sample of flour (F2), four bottles were prepared in the usual way. Two were kept over night in an icebox in a temperature of 5° C. The other two were kept for 16 hours in a thermostat at 30°C. Next morning the color values of these extracts were made. The bottles kept in the ice-box gave an average value of 1.75; the bottles kept at 30°C., a value of 2.36. The same flour gave (Table VIII) after 16 hours, a value of 2.04; and after 40 hours, a value of 2.37. This test shows that the temperature has a great influence on results, and that the same results can be obtained in 16 hours at 30°C. as in 40 hours at room temperature.

Conclusions

The rapid gasoline extraction method described by Coleman and Christie (1926) can easily be carried out by using a shaking machine. This permits the use of tightly closed glass-stoppered bottles and eliminates all evaporation.

The colorimeter designed by Kent-Jones and Herd (1927) is less tiring to the eye than the Duboscq and having a constant source of light makes the worker independent of the daylight.

We prefer the Duboscq colorimeter to the Kent-Jones, however, because with the latter a reading takes much more time and is no more accurate.

The principle of the Kent-Jones colorimeter, which involves the use of standard solutions of different concentrations, is a source of errors. The use of this principle is only permissible if the unknown solution has the same components as the standard solution. If solutions of different composition are compared, several precautions must be taken to avoid this error.

This error does not appear in separate gasoline value tests of the same sample of flour because in each instance the same error is made. But results are very misleading when a flour is compared with another flour that has twice the amount of carotinoid pigments. The last flour will give a gasoline value that is between two and three times as high.

We have shown that this error is probably due to the differences in H-ion concentration of the standard solution, because it could be largely corrected by the use of buffer solutions instead of distilled water.

The use of an entirely different standard solution made of organic dyes, as used by Sprague (1928), also avoids this error. We have a favorable impression of this standard solution because it has a tint of yellow that matches the color of the gasoline extract much more closely than any of the inorganic standard solutions.

An objection to the organic dye standard solution is that not all samples of the organic dyes have the same purity and therefore not the same color value. If this difficulty could be overcome, for instance, by having available official samples of these dyes, standardized against pure carotin solutions, these organic standard solutions would certainly be an improvement over the inorganic standard solutions.

We agree with Jørgensen (1927) that it is advisable to use buffer solutions of a known H-ion concentration for the preparation of the official standard solutions of potassium chromate.

The extraction with alkaline methyl alcohol does not, in the 16 hours specified by Kent-Jones. give a complete extraction of all the coloring matter soluble in this solvent. Only after approximately 40 hours are constant results obtained.

We found it necessary to shake the extract thoroly at the end of the extraction period, a fact to which the author does not call attention in his book, "Modern Cereal Chemistry." In an earlier

publication, however, he gives directions to shake for 16 hours. We found that this gives the same results as standing for 16 hours and shaking after this period.

We found that the temperature at which the extraction is made has a decided influence upon the results obtained. An extraction of 16 hours at 30°C. gives the same results as an extraction of 40 hours at room temperature. We therefore think that there is a possibility of improving the method by using a higher temperature for a short period. Lack of time has prevented our completing these tests; we hope to be able to publish them in the near future.

We find the same relation between the methyl alcohol value and the ash content of flour as was observed by Kent-Jones and Herd. Our tests, in which different amounts of bran were added to a sample flour, show that there is a direct relation between the bran content of the flour and the methyl alcohol value.

Literature Cited

American Association of Cereal Chemists
    1928 Methods of analysis of cereals and cereal products.
Bailey, C. H.
    1925 The chemistry of wheat flour. Chemical Catalog Co. New York.
Coleman, D. A. and Christie, Alfred
    1926 A rapid method for determining the gasoline value of flour and wheat. Cereal Chem. 3:84.
Jago, W.
    1911 (1921) The technology of breadmaking. Northern Pub. Co., Ltd. Liverpool.
Jørgensen, Holger
    1927 Use of chromate solutions as comparison standards for the determination of the gasoline color values. Cereal Chem. 4:468.
Kent-Jones, D. W.
    1927 Modern cereal chemistry. Northern Pub. Co., Ltd. Liverpool.
Kent-Jones, D. W. and Herd, C. W.
    1927 A numerical expression for the color of flour. Analyst 52:443-452.
Monier-Williams.
    1912 Report to the local government board on the nature of the coloring matter of flour and its relation to processes of natural and artificial bleaching. Rept. Local Gov. Board, Pub. Health and Med. Subjects, N. Series No. 73.
Palmer, L. S.
    1922 The carotinoids and related pigments. Chemical Catalog Co., New York.
Priest, Irwin G.
    1928 Color sensitivity. Oil and Fat Indust. 5:63.
Sprague, Howard B.
    1928 A convenient method of measuring quantities of chloroplast pigments. Science 67:167-169.
Willstätter, R. and Stoll, A.
    1913 Untersuchungen über Chlorophyll, Methoden und Ergebnisse, Berlin.
Winton, A. L.
    1911 Color of flour and a method for the determination of the gasoline color value of flour and wheat. U. S. Dept. Agr. Bur. Chem. Bull. 152:114-116.

HOW THE EXPERIMENTAL BAKING TEST HAS DEVELOPED

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Baking tests are now generally conceded to be the most important means of judging flour quality. It is impossible to say when the first baking test was made. Altho written records of such tests extend back hardly a hundred years, it is possible that for centuries previous some type of more or less crude baking tests was resorted to, especially by the purchaser of large quantities of flour.

A search through available literature has failed to reveal any description of baking tests prior to the middle of the nineteenth century, altho methods are described as early as the latter part of the eighteenth century. Maurizio (1903) in his work "Getreide, Mehl und Brot," which includes a valuable summary of the development of the baking test, mentions that tests were made about 1830, but does not describe them or give references. It is very likely that the increasing preponderance of wheat over rye, as well as the introduction of scientific breeding experiments and the more exacting requirements of the miller and flour buyer, were largely responsible for the development of organized methods for testing wheat flour.

The earliest reference to baking tests that we have been able to find concerns the experiments of Heeren (1854), who in 1852 conducted some carefully controlled investigations of the yield of bread from different flours. Maurizio (1903) mentions even earlier investigations of yield by Accum, Prechtl, Dumas, and others.

Some years previous to this, Boland (1849) had proposed the use of his "Aleurometer" for measuring the baking quality of flour through determining the strength of its gluten, and had advanced what seemed to be convincing proof of the reliability of his method, which was widely used for a good many years. But in the course of time doubters arose, notably Balland (1883, 1894), who criticized the method thoroly, having found that the results obtained by its use were not indicative of baking quality.

Baking tests on a laboratory scale were made by Maercker and Besseler (1887), who concluded that neither the amount nor the strength of the gluten was directly related to baking quality.

It is interesting to note that Graeger and v. Bibra (1861) expressed their doubts as to the reliability of baking tests made by commercial bakers, on account of the lack of uniform conditions. Nearly fifty years later, Kosutany (1907) wrote that laboratory baking tests could not be depended on except for approximate results, and that it would be far more suitable to submit the flour to a technically trained baker for perhaps a week's testing under commercial conditions. Not long afterward another European investigator wrote that the carrying out of baking tests in a manner different from that used in commercial practice does not lead to reliable results, and that only in exceptional cases can these tests be of value.

Very few references to flour testing in America can be found for this early period. Clifford Richardson (1884) conducted baking tests of flours of different grades from different parts of the country, concluding that "the yield is dependent on the physical conditions of bread making, and not to a large extent on the chemical composition of the wheat; further, that the percentage of gluten has but little effect on the yield."

Jago (1911) states that in all American systems of flour testing that have come to his attention, the baking tests are made on tinned bread.

In 1886 a flour testing laboratory was started in Minneapolis by A. W. Howard, a former miller, who for some time had been much in demand throughout the country as a good judge of flour. The first reports from this laboratory were based on loaves baked in a commercial shop, but a laboratory method was soon developed. The oven used had a rotating shelf and held only one loaf. At first a commercial loaf was made, but later, probably about 1895, a pan with high sides was adopted.

As stated above, the determination of baking quality through measuring the expansion of washed gluten gradually fell into disfavor. A number of investigators attempted to devise laboratory baking tests to supplant it. Pekar (1882) was the first to report determinations of loaf volume.

A method originated by Kreusler in 1887 aimed to produce a small loaf, baked under conditions as nearly as possible like those existing in a large bake oven, and having, in his words, "all the properties of a normal oven-product." The small oven employed consisted of an oil-bath over which was mounted a rotating shelf, provided with openings to receive small baking pans. The dimen-

sions of the pans are not stated. Kreusler specified a constant amount of water for all doughs, but Maurizio (1903), in describing the apparatus and its use, stated that the amount of water used should be that necessary for a dough of normal consistency, and also said that on account of the variations in judgment among different operators, the amount of water used may vary as much as 2-10 cc. per 100 g. of flour.

A somewhat similar method was worked out by Kunis (1898). He constructed an apparatus called the "Farinometer," which employed small doughs made from 30 g. of flour. Two equal portions of the dough, one with sodium bicarbonate and one with tartaric acid, were baked in metal cylinders placed in a small oven heated by an alcohol lamp, and their volumes were compared. This apparatus is much similar to that described by Jago (1895) and others.

Sellnick (1902) described a somewhat different apparatus called the "Artophon" for testing flour. A dough was made with yeast or baking powder and baked on a flat plate over a water bath heated by a spirit flame and covered by a bell jar. No pans were used.

Maurizio (1902), after testing the methods of Kreusler, Kunis, and Sellnick, concluded that except for certain drawbacks as regards heat distribution the Kreusler apparatus was the most reliable and gave the best comparative results. The other methods he characterized as "quite primitive." He therefore set about to devise an apparatus of his own, provided with more accurate means of controlling the temperature and heat distribution. The oven used was a double-walled, copper drying oven, well insulated with asbestos paper, and so constructed as to give uniform heat distribution. Instead of baking cylinders, small shallow pans with sloping sides were used. They measured 3 cm. in height, 8.5 cm. in diameter at the top, and 5 cm. in diameter at the bottom. Ninety g. of flour was the amount usually employed for baking tests. The amount of water was varied to suit the flour.

A method much like this was reported by Maercker (1893) from the Agricultural-Chemical Experiment Station at Halle, Germany, where baking tests were first made in 1886. At first small hearth loaves (semmel) were baked, using a bakers' oven, but later a cylinder 7.5 cm. in height and 6 cm. in diameter was adopted. In the earliest tests the same amounts of water were used for all flours, but "experiments soon confirmed the common

observation of bakers that different kinds of flour require different proportions of water to make a dough of given consistency."

Apparently these methods, in which the baking quality of flour was judged from a small loaf baked from 100 g. of flour or less, were slow in gaining popularity in the United States and Canada. Saunders (1907) described a method in which only 50 g. of flour are used. The loaves were baked in small shallow pans, of nearly the same dimensions as those used by Maurizio. The oven was heated with gas and provided with a rotating shelf to insure uniform baking conditions. Small loaves were made to economize flour and time and to permit the repeated baking of each sample studied. Enough water was used to bring the dough to the desired consistency in each case.

Werner (1925) described a method based on that of Maurizio (1903) and designed to give concordant results within a small factor of error when performed in duplicate or triplicate. Werner and Herman (1928) summed up the background of the "differential test," stating its advantages and its purpose.

Methods in which a small baking cylinder is employed are used in some parts of the United States, especially for testing soft wheat flours.

Fornet (1925, 1926) described a device called the "Mehlometer," consisting of a combination fermentation cabinet and oven and a set of small square pans and other essentials for producing a small loaf from 100 g. of flour, having the same cross-sectional area as the commercial pan loaf.

To return to American methods: Harper (1892) reported baking tests made "to determine (1) The amount of flour necessary to make the best bread with a definite quantity of yeast liquid. (2) The strength of the flour as determined by the dimensions of the loaf made from a definite quantity of flour and yeast mixture. (3) The absorption and retentive capacity of the flour as determined by the weight of the bread made with a definite quantity of yeast mixture and flour, and (4) the quality of the bread as determined by its color and texture."

Senn (1896) concluded from baking tests that the amount of flour necessary to make bread varies with its gluten content.

A few years later Harcourt (1901) described tests of Ontario wheats for bread making. The tests were made by experienced bakers; a commercial loaf being produced. Apparently the treatment was adapted to suit the individual flour.

Snyder (1904, 1905) described the testing of wheat flour for commercial purposes by means of large-scale commercial tests, in which "the various ingredients were exactly proportioned and the fermentation and mechanical manipulations were carried out under standard and uniform conditions." The amount of water was varied to suit the flour.

Thatcher (1907a, 1907b) and Wessling (1908) describe the "Koelner system for the scientific testing of flour," which included a sponge and a baking test; the sponge test being relied upon to show the strength of the flour, and the baking test the color and texture.

A method producing a one-pound loaf was reported by Ladd (1908). A constant amount of water was used and the flour was varied. Later Ladd (1910) adopted the reverse procedure. All doughs were handled the same, the amount of water being the only variable.

Another method for producing a commercial loaf is reported by Stewart and Hirst (1910). Here also the absorption was varied. The loaves were proofed to the maximum. Bailey (1916) presented a method for determining the strength and baking quality of wheat flour, using a mechanical device called the "expansimeter," in conjunction with a baking test.

Many other methods might be cited here from experiment stations and other laboratories throughout the country, but as many of these are essentially similar they have been omitted. The majority of the methods in use for the testing of flour in American laboratories are designed to produce a one-pound loaf as much like the one-pound loaf of commerce as possible. Fitz (1924) reported a survey of formulas and methods of procedure for experimental baking tests in use in about fifty laboratories. These formulas were all of the type designed to produce something resembling a commercial loaf, but the laboratories were using a great variety of formulas and methods of procedure.

In some laboratories the high pan is in use, and has been in use for some years. Usually when such a pan is used the object of the test is to produce as large a loaf as possible, i.e., to ferment and proof the dough to the maximum. The resulting bread, while much coarser in texture than the commercial product, gives a large surface from which to judge color, and those who are experienced in the use of this method claim that it reveals information about

the strength, color, and tolerance of a flour that otherwise would remain hidden.

Dunlap (1926) pointed out the significance of the human element in dough handling, concluding that a test bake "made in accordance with the character of the flour and in such a manner as to produce the best loaf of which the flour is capable" is the only way of determining the actual quality of flour.

Numerous questions were raised by Blish (1926) with regard to the meaning and purpose of the baking test. He stated that cereal chemists ought to agree on the meaning of the terms "baking quality" and "baking strength," also the exact nature of the information the test is expected to furnish. He felt that an attempt might be made to establish an official or approved method in which the formula, temperatures, and dimensions of pans were standardized.

Herman and Hart (1927) described a large number of tests made with small loaves, using 100 g. of flour, and discussed the effect of variations in ingredients, apparatus, and conditions. Blish and Sandstedt (1927) advanced a "general discussion of factors upon which one may substantially base interpretation of baking tests conducted according to the fixed type of procedure," advocating the use of the differential test as an added modification for taking account of the action of oxidizing agents.

Various types of baking tests were discussed by Haas (1927), who pointed out the advantages of a test purported not only to give the best loaf possible, but also to give information as to "the ability of flour to stand abuse and to give good results over more or less widely varying conditions."

Blish (1927) discussed the function of a "standard test" of a "raw" material, stating the advantages of a carefully controlled test using a fixed procedure, and the shortcomings of a variable test. In the report of the Committee on Standardization of the Experimental Baking Test, Blish (1927) presented a definite recommendation of the fixed type of procedure. Later, Blish (1928), as chairman of the committee reported the detailed baking procedure proposed by the committee.

To summarize, the literature shows that the first baking tests on record were made for the purpose of determining the yield of bread, and it was not until toward the close of the last century that definite methods were developed for testing flour with a view to de-

termining its expansion, color, and other characteristics as shown by the baked loaf.

Judging from the results reported, the tendency among European workers has been to develop a test in which a small quantity of flour (100 g. or less) is used to produce a loaf having characteristics analogous to those of the large loaf of commerce. In practically all cases a uniform procedure is employed, but the amount of water is varied for different flours.

In America two main tendencies can be distinguished: (1) The attempt to ascertain the maximum volume that a flour will yield, in some cases with the addition of stimulants to a large percentage of yeast. (2) The attempt to vary the baking procedure, particularly fermentation time, in such a way as to produce an ideal commercial loaf. The use of small quantities of flour yielding loaves substantially smaller than commercial loaves has not been so widespread, altho some laboratories have employed this type of test for some years. Numerous workers have found it difficult to arrive at definite conclusions from single baking tests. The biochemical changes taking place during the fermentation of a dough are so many and so varied that replicate determinations must be considered necessary.

The tendency to establish fixed conditions for the baking test, with flour as the only variable, is exemplified by the work of the baking test committee of the A. A. C. C., as well as by the reports previously cited here.

Literature Cited

Bailey, C. H.
  1916 A method for the determination of the strength and baking qualities of wheat flour. J. Ind. Eng. Chem., 8:53-57.
Balland, A.
  1884 Journ. de pharm. et de chimie, 5e serie, tome VII. (Citation by Maurizio, 1903, p. 287. Original not seen).
  1894 Recherches sur des bles, les farines, etc. p. 17. Paris. (Citation by Maurizio [1903]. p. 287. Original not seen).
Blish, M. J.
  1926 The problem of standardizing the experimental baking test. Cereal Chem. 3:216-222.
  1927a A rational basis for the standardization of the experimental baking test. Cereal Chem. 4:149-155.
  1927b Report of committee on standardization of the experimental baking test. Cereal Chem. 4:299-310.
  1928 Standard experimental baking test. Report of Committee of the American Association of Cereal Chemists. Cereal Chem. 5:277-300.
Blish, M. J. and Sandstedt, R. M.
  1927 Factors affecting interpretation of experimental baking tests. Cereal Chem. 4:291-299.

Boland
    1849 Verfahren des relativ Tauglichkeit des Weizenmehls zum Brod-
        backen mittelst des Aleurometers zu bestimmen, eines von der
        Bäckermeister Boland in Paris erfundenen Instruments. Dinglers
        Polytech. J. 111:117-123.
Dunlap, F. C.
    1926 The problem of test bakes, with a discussion of certain of their
        chemical and physical aspects. Cereal Chem. 3:201-215.
Fitz, L. A.
    1924 Formulas and method of procedure for experimental baking tests.
        Cereal Chem. 1:251-260.

    1925 Report of committee on standard formula and method of proce-
        dure for experimental baking tests. Cereal Chem. 2:232-235.
Fornet, A.
    1925 Beurteilung der Getreidemehl und der Backzutaten durch das
        Backprozess im Laboratorium. Chem. Ztg. 49:347-348. (Chem.
        Abs. 19:2539).
    1926 Die Theorie der praktischen Brot und Mehlbereitung, p. 231.
        Berlin.
Graeger and v. Bibra
    1861 Die Getreidearten und das Brot. Nürnberg.
        (Citation by Maurizio [1903], p. 297. Original not seen).
Haas, L. W.
    1927 Interpretation of baking tests. Cereal Chem. 4:389-394.
Harcourt, R.
    1901 The comparative values of Ontario wheats for bread-making
        purposes. Ontario Agr. Col. Bull. 115.
Harper, D. N.
    1892 Milling and baking tests. Minn. Agr. Expt. Sta. Bull, 23. pp.
        141-146.
Heeren
    1854 Ueber die Menge Brot, die man aus einer gegebenen Menge Mehl
        erhält. Chem. Zentralbl. 25:301.
Herman, R. S. and Hart, V. M.
    1927 Some factors influencing the experimental baking test. Cereal
        Chem. 4:157-183.
Jago, Wm.
    1895 The science and art of breadmaking. London.
Kosutany, T.
    1907 Der ungarische Weizen und das ungarische Mehl. Budapest.
Kreusler
    1887 Zur Ermittlung der Backfähigkeit des Mehles. "Die Mühle" Nr.
        35. (Citation by Maurizio [1903], pp. 287, 295-299. Original not
        seen).
Kunis
    1898 Wie untersucht man Getriede und Mehl? Leipzig. (Citation by
        Maurizio [1903], p. 293. Original not seen).
Ladd, E. F.
    1908 Milling and baking tests. No. Dak. Agr. Col. Bull. 82.
Ladd, E. F., and Bailey, C. H.
    1910 Wheat Investigations. No. Dak. Agr. Col. Bull. 89.
Maercker, M. and Besseler, O.
    1887 Versuche über den Anbauwert verschiedener Sorten von Winter-
        und Sommerweizen. Magdeburger Zeitung. (Citation by Maurizio
        [1903], p. 288. Original not seen).
Maercker, M.
    1893 The Agricultural Experiment Station at Halle, Germany. Expt.
        Sta. Rec. 5:372-374.

Maurizio, A.
1902　Die Backfähigkeit des Weizenmehls und ihre Bestimmung. (Description of bake oven). Thiels Landwirtschaftl. Jahrbücher, 31, 1902. (Citation by Maurizio [1903], p.300. Original not seen).

Maurizio, A.
1903　Getreide, Mehl und Brot. Verlagsbuchhandlung. Paul Parey. Berlin.

Pekar
1882　Weizen und Mehl unserer Erde. (Citation by Maurizio [1903]. Original not seen).

Richardson, Clifford
1884　An investigation of the composition of American wheat and corn. U. S. Dept. Agr., Bur. Chem., Bull. 4.

Saunders, C. E.
1907　Quality in wheat. Center. Expt. Farm, Ottawa. Bull. 57.

Sellnick
1902　Das Artophon; Anweisung zu dessen Gebrauch. Leipzig-Plagwitz. (Citation by Maurizio [1903], p. 293. Original not seen).

Senn, Marie B.
1896　Bread making. No. Dak. Agr. Expt. Sta. Rept. pp. 43-46. (Expt. Sta. Rec. 9:785).

Snyder, Harry
1904　Wheat and flour investigations. Minn. Agr. Expt. Sta. Bull. 85.
1905　Testing wheat flour for commercial purposes. J. Am. Chem. Soc. 27:1068.

Stewart, R. and Hirts, C. T.
1910　The influence of the combined harvester on the value of the wheat. Utah Agr. Expt. Sta. Bull. 113.

Thatcher, R. W.
1907a Wheat and flour investigations. Wash. Agr. Expt. Sta. Bull. 84.
1907b A comparison of various methods of estimating the baking qualities of flour. J. Am. Chem. Soc. 29:6.

Wessling, Hannah L.
1908　Examination of flour with practical tests. 13th Bien. Rep. Bureau of Labor and Industrial Statistics. Part V. pp. 810-814. Madison, Wis.

Werner, E. E.
1925　The baking test. Cereal Chem. 2:310-314.

Werner E. E. and Herman, R. S.
1928　The differential baking test. Northwestern Miller. 5:1216.

THE MECHANICAL METHOD OF MODIFICATION OF DOUGH[1]

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(Read at the Convention June, 1928)

The mechanical method of modification of dough has been described by Swanson and Working (1926). Briefly, the method is to treat dough made according to usual formulas in a specially constructed mixer that gives the dough a very severe mechanical treatment. The dough is then placed at once in the baking pan, allowed to rise a definite amount, and baked. The use of a small amount of lactic acid in the formula is helpful in that a small variation in the correct amount of water will have less influence on the results. The action of the mixer on the dough is so severe that no gluten can be washed from dough treated for five or more minutes.

The method has been found most useful in the study of characteristics or qualities of wheat varieties. Some varieties always produce good bread by this method; other varieties, poor bread. The results of four years' work are very consistent. It makes little difference where a certain variety is grown or in what year. Superhard will always give bread of heavy, coarse texture and small volume, similar to Durum. A genuine Turkey or Marquis will always give bread of light, fine texture and large volume. The manner in which the dough develops in the mixer also tells much about the quality. Doughs from flours that as a rule produce unsatisfactory bread develop very quickly and then break down, or become slack. Doughs from flours that as a rule give satisfactory bread, develop more slowly and do not break down or become slack. With strong flours it takes longer for the water to penetrate into the protein particles and form gluten, but such flours have more resistance against mechanical action.

The mechanical method of modification has proved its value in connection with wheat improvement work at this station by its sure detection of any weakness inherent in the gluten so far as that is related to the strength of the colloid filaments formed by the protein particles. We have found that this method will reveal

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[1] Contribution No. 35, Department of Milling Industry.

greater differences in wheat varieties than the conventional baking tests.

Evaluating commercially important wheats is a different matter from testing varieties from the plant-breeding plots. In this connection it is necessary to consider three questions:

1. Does this method show as great differences or greater in flours from commercially important wheats as the methods commonly used by cereal chemists in their laboratories?

2. How are the differences in flour obtained by the mechanical modification of dough correlated with the results secured in the commercial bakeshop?

3. Is the method unnecessarily severe? That is, if a flour gives poor results by this method should it be classed as weak?

The three most important hard winter wheat varieties grown in Kansas are Blackhull, Turkey, and Kanred. The comparative qualities of these have been discussed by Salmon, Swanson, and Laude (1927).

Table I gives a comparison of average results obtained on these three varieties with a common fermentation method and the mechanical method of modification.

TABLE I
COMPARISON OF MECHANICAL METHOD OF MODIFICATION AND CONVENTIONAL METHOD
IN STUDY OF THREE LEADING WHEAT VARIETIES

Year	Variety	No. of samples	Protein	Abs.	Loaf volume	Texture
			%	%	cc.	%
		Fermentation Method				
	Blackhull	40	11.7	61	1894	91
1923	Turkey	40	11.4	61	1893	90
	Kanred	40	11.9	61	1874	90
	Blackhull	49	11.3	53	1699	90
1924	Turkey	49	10.9	54	1705	91
	Kanred	49	10.9	54	1677	89
		Mechanical Method				
	Blackhull	47	12.1	56	1680	89
1925	Turkey	47	12.1	60	1860	95
	Kanred	47	12.3	59	1898	96
	Blackhull	27	13.8	67	1701	85
1926	Turkey	27	13.9	71	1838	93
	Kanred	27	13.7	71	1923	94

The outstanding facts to be noted from these results are that with the conventional method no essential differences can be detected in the three varieties, but with the mechanical method of modification Blackhull is shown to be decidedly inferior to the

other two varieties. This is indicated by absorption, loaf volume, and texture. The inferiority in these characteristics was obtained on Blackhull with remarkable consistency. Apparently it made no difference in what year or in what locality in the southwest the wheat was grown, for samples of these varieties grown in 1924, 1925, 1926, and 1927 all showed consistent results.

In the fall of 1927, a small lot of Blackhull wheat grown near Manhattan, Kansas, was milled in the college mill. Tests made with the mechanical method of modification of dough showed typical Blackhull characteristics. One barrel of this flour was baked in a local bakery in comparison with a barrel of college flour milled from a typical Turkey wheat grown in Lincoln County, in the center of the Kansas wheat belt. No important differences were observed in two lots of bread, showing that a satisfactory bread may be made in a commercial bakeshop from a flour that gives very poor results with the mechanical method of modification. As nearly as could be estimated, the treatment of the high-speed dough mixer of the baker was equivalent to about one and a half minutes with the laboratory machine used for mechanical modification. With this amount of mixing and the usual amount of fermentation, a satisfactory bread was obtained in our laboratory from the same Blackhull flour. This shows that flour from Blackhull may be satisfactory with suitable methods of handling and also that it does not resist as much mechanical treatment as flour from Turkey wheat.

Several other experiments have demonstrated that a satisfactory bread may be made from other commercially important wheats that have been shown to be inferior by the method of mechanical modification. Hence it follows that for such wheats the method is unnecessarily severe. It does, however, show greater differences in some commercially important flours than the commonly used methods. How to evaluate these differences is not so clear.

The best way to test a new and unknown variety of wheat is to compare it with an older and tested variety. A new winter wheat named Tenmarq has been developed at the Kansas State Agricultural Experiment Station. It is a selection from crosses of Kanred and Marquis spring wheat. It has very excellent agronomic qualities. Before distributing this variety, it was desired to have comparative tests made. Tenmarq and Turkey wheats, both grown in Brown County, northeastern Kansas, were milled on the

college mill. Samples of flour were sent to cereal chemists, two of whom were connected with flour mills, two with agricultural experiment stations, and one with a large bakery.

The following statements taken from the reports show a wide variety of opinion:

Chemist A. "The grain structure of Tenmarq is unsatisfactory in comparison to the Turkey and clearly indicates an inheritance of Marquis characteristics. The Tenmarq carried a slightly greater resistance to mechanical modification and to oxidizing agents than the Turkey."

Chemist B. "Neither baked a satisfactory loaf. Both had miserably poor shell top. There was really no difference between them. The loaves had a shell top, which indicates lack of strength of gluten. Both loaves were good in color and texture, and were more or less dull grayish white instead of creamy white."

Chemist C. "We would rate both samples as very good, strong bread flours. Both produced loaves of good volume and good texture under the system we use, which is rather severe. They showed good fermentation tolerance. A comparison of texture would indicate (to us, at any rate) slightly greater strength in Turkey than in Tenmarq. As to their resistance toward oxidizers, we would again give slight preference to Turkey altho both indicated good tolerance in the oxidizer test."

Chemist D. "There appears to be a significant difference in the average loaf volume of the two series in favor of the Turkey Red. The probable error of their difference was found to be approximately one-eighth of the difference, thus justifying the conclusion that there is a real difference."

Chemist E. "The stability or range of time over which good bread could be produced with Tenmarq was much greater than that for Turkey. (This is a quality in a bread flour.) The grain of Tenmarq bread was finer than that of Turkey. The texture of Tenmarq bread was firmer than that of Turkey. The break and shred of Tenmarq loaves was smooth, thus pro-

ducing a nice appearing commercial loaf. A larger loaf with even better inside appearance was possible with Tenmarq then with Turkey. Tenmarq appeared to have many characteristics similar to Turkey but the good qualities were much more intensified in Tenmarq. Tenmarq failed to carry by 1.4 per cent the absorption carried by Turkey, but this single point is far outweighed by its other good points and might even be the same as Turkey on another sample. This 1.4 per cent might be in part accounted for in the technic of baking."

It would be rather difficult for the plant breeder to judge from these replies which was the better of these two wheats. He would conclude that cereal chemists are far from agreeing on evaluating results obtained on flours. Could an experiment station decide on such a basis that a new wheat like Tenmarq is one that will give satisfaction to the milling and baking trade? The method of mechanical modification showed Tenmarq superior to Turkey, hence on the basis of this test there is no doubt.

This situation indicated the advisability of having a conference of cereal chemists. To this conference were invited all those who had co-operated in the tests just described as well as several others. The conference was held at Manhattan on February 13 and 14. Three of the co-operators and five other chemists were present. Each chemist was asked to send in two samples of flour, one representing a weak flour and the other a strong flour, based upon determinations made in his own laboratory. On the first day E. B. Working baked one loaf from each of the samples of strong flour, and one from the weak, using the method of mechanical modification. At the same time, each chemist baked his own samples by the method he preferred. The majority used, as far as possible, the method recommended by the American Association of Cereal Chemists. A few used the method employed in their own laboratory, some mixed the dough by hand, others used a machine capable of giving any degree of mixing desired.

The second day was devoted to judging the bread and discussing results. Each chemist first discussed the results obtained on his own flour by his own baking and then compared these with the results obtained by the method of mechanical modification. These discussions very clearly brought out three points:

1. The variations in methods employed by the different chemists.
2. The divergent opinions as to the interpretation of the results of the specific baking tests.
3. The difficulty of getting anything like a unanimity of opinion regarding the merits of any particular lot of flour.

In order that as fair an impression as possible might be reported of the results of the meeting, the chemists were asked to reply formally to four questions. The following are the questions together with extracts of the replies:

1. What is your judgment on the value of the results obtained by the mechanical modification of dough in comparison with other methods?

The following are some of the principal opinions expressed: "It seems that the interpretation of results is decidedly contrary to that obtained by the more common means of conducting experimental baking tests. It is my reaction that the mechanical modification method deals only with colloidal properties and gives no indication of fermentation tendencies. There seems to be some correlation between the resistance to mechanical modification and the resistance of oxidizing agents." "It is my belief that the value of the results obtained by the mechanical modification of dough in comparison with other methods places it far ahead of the ordinary procedure for baking employed in the average mill laboratory. As to whether it outranks the method now under consideration by Dr. Blish's committee, I am not prepared to say." "The value of the results obtained by the mechanical modification of dough is just one. It tells how much resistance to mechanical punishment any dough will stand." "This is strictly a test of gluten strength. For this purpose I regard it as decidedly more definite and informative than the viscosity test."

2. Can the differences obtained by this method be correlated with values based on bakeshop practice?

Some of the answers were as follows: "I do not see what practical value can be based on the test aside from a measurement of the resistance of dough to mechanical modification." "The differences can be correlated only as to mixing time." "With careful work any baking method can be correlated with values based on shop practice. Results obtained in the laboratory will not be the same as those obtained in the bakeshop, but they will parallel

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each other with a perfectly definite factor differential provided the laboratory method is sufficiently accurate."

3. Does the method treat the dough with more severity than necessary?

There was more unanimity in the replies to this question than to the first two. "As you use it, the mixing is too severe." "It is excessive." "The seven-minute treatment is entirely too severe and no flour should be rejected because it does not stand such severe treatment." "Treatment more severe than will be found in the average bakeshop should not be used." "The mechanical modification apparently places stress upon the mechanical development and conditioning of dough." "More upon the mechanical than upon either the chemical or enzymatic methods of conditioning the dough."

4. How does the efficiency of our mixer compare with others for use in making any kind of baking tests?

This question was included because a few chemists have from time to time expressed a desire to have one of these mixers for experimental work. The purpose of this question was to find out if the opinions of the value of the machine were such that any manufacturer would be warranted in making a certain number. There was a unanimity of opinion in regard to the efficiency of the machine, as any degree of mixing desired can be given the dough. "It is more efficient than any other laboratory machine now on the market." "It is a very flexible machine, being capable of running on slow speed as well as high, or any intermediate speed desired." However, only two chemists stated definitely that they desired to have one in their own laboratory.

The following summary statement was given by one of the attending chemists: "This is strictly a test of gluten strength. For this purpose I regard it as decidedly more definite and informative than the viscosity test. In a comparative way, it will surely indicate inherent tolerance or lack of tolerance toward severe mechanical mixing. This is certainly a factor of importance in commercial baking. Before recommending a new wheat variety, or in discussing the relative merits of established varieties, it is important to know the wheat's status in regard to this factor. The results seem to be correlated with a specific character of the gluten, and they are consistent."

The following conclusions may be drawn from these expressions: The mechanical method of modification is much more

severe than any flour would be expected to withstand in average industrial practice. It would therefore not be advisable to condemn a flour by its failure to give good bread by this method. It would be admissible to say that the use of the method was more restricted, or that methods of commercial baking would have to be modified to suit a flour that showed itself weak by this method. The greatest value of the method seems to be in revealing certain weaknesses in flour.

That the method does not indicate fermentation tolerance was a general criticism. Fermentation tolerance is taken to mean the time the dough can lay over after it is properly developed, and still produce good bread. It does not refer to the total length of the fermentation period, as this may be longer or shorter with equally good flours and the period would have to be adjusted for the different flours. One hour is a good fermentation tolerance, that is, when a dough is developed so as to produce a good bread after three hours fermentation, it will also give a good bread after four hours.

Does the mechanical method of modification indicate fermentation tolerance? At this point it might be well to consider briefly what takes place during the fermentation of bread doughs. The acids produced by yeast action would enter or be absorbed into the gluten structure. This would cause a swelling or an incipient dispersing action resulting in greater pliability and extensibility. Hence this effect of fermentation would be toward larger loaf volume. The alcohol would weaken the surface tension of the water films on the gluten strands and the starch particles. This would lessen the strength of the cementing action of water and to that extent decrease the resistance to the expanding gas bubbles. As gliadin is alcohol-soluble, there would be a slight incipient gluten dispersion similar to the effect of acid. The main effect of both alcohol and acid would be to increase pliability with resulting larger loaf volume. There must be a nice balance of these factors. If the pliability or extensibility of the dough is too great, the bread will be coarse; if too little, the loaf volume will be small and the texture heavy.

Another effect of fermentation is the disappearance of sugar. For this reason diastatic activity has been at times considered one of the factors in flour quality. This is of secondary importance, as whenever the diastatic activity is low this can be remedied by the addition of more sugar or malt. Lastly, there might be some proteolysis,

but this does not seem to extend so far as to set free any amino groups. It might produce some so-called metaproteins, that is, products resulting from the breaking down of the large protein molecules. This might be, in part at least, a reason for loss of gas-retaining capacity in over-fermented dough.

We do not seem to know very clearly the function of the ash elements. In conventional terms we say that the buffer effect is somewhat proportional to the ash content. The surprising thing is the large effect from a very small amount of ash. The ash elements are apparently in combination with the protein and other organic compounds. The presence of such combinations or compounds seem to counteract the effect of the acids and possibly of the alcohol.

In studying the question of quality, we must consider (1) the factors inherent in the flour itself, (2) the ingredients used in mixing the dough, (3) the methods of mixing, (4) the temperature of fermentation, and (5) the by-products of fermentation. All but the first are under the control of the baker. It seems, therefore, that in testing a flour primary consideration should be given to the factors inherent in the flour itself and the mechanical method of modification has been developed with this primary object in view, namely, to test the factors which determine gluten quality. Fermentation tolerance is very intimately related to the ingredients used, the temperature, and the by-products. Just how much it is related to the factors inherent in the flour itself is not so clear.

Going back to the question, Does the mechanical method of modification indicate fermentation tolerance, only an indirect answer can be given. The method gives excellent results from flours made from standard wheats, such as Turkey and Marquis. It always gives a poor loaf from certain white wheats that are considered weak, and also from Durum and Superhard. Flour from Blackhull wheat as a rule produces unsatisfactory results with the mechanical method of modification. Flour that lacks fermentation tolerance is unsatisfactory in the trade. One of the most prominent millers in Kansas City made this statement to the writer, "Blackhull is a satisfactory wheat if the baker will adjust his period of fermentation to suit this flour. If he does not do this, there is trouble." A number of statements to the same general effect have been received. Blackhull gives poor results with the mechanical method of modification and since some important trades think it lacking in fermentation tolerence, it seems that there is at least

some relation between resistance to mechanical action and fermentation tolerance.

With the mechanical method of modification, the quality of protein seems to be more important than the quantity. Loaf volume and texture do not correlate with the protein content. Certain wheat varieties, as has been stated, always give a poor loaf, others a good loaf, no matter what the protein content. This is one reason why, in the recent conference reported in this paper, the mechanical method did not show such differences between strong and weak flour as the methods used by the attending chemists.

The conventional methods used in baking tests seem to have been developed along lines that favor the average flour of a certain class. With these methods, the loaf volume correlates better with protein content. At least this is true for the commercially important wheats. With the method of mechanical modification, quality seems to be more important than quantity. To what these quality differences are due is not so clear. They apparently reside in the colloid properties of the protein particles which form gluten when water is added to flour. Such qualities are inherent in the flour itself.

Just how to interpret all the results obtained with the mechanical method is not known. However, results obtained on commercially important wheat varieties accord in general with the opinions of the trade.

Summary

1. The mechanical method of modification has shown its greatest value in revealing weaknesses in new and unknown varieties of wheat.

2. It is more severe than necessary for testing commercially important wheats, that is, flours that give poor results with this method may give satisfactory results in the commercial bakery if suitable procedures are followed.

3. There is a large variation in testing methods employed by cereal chemists, and the opinions as to the interpretation of the results of specific baking tests are very divergent. For these reasons it is almost impossible to get anything like a unanimity of opinion regarding the merits of different flours.

4. The machine used in our laboratory is more efficient than any one now on the market. Any degree of severity of mixing may be obtained and it is uniform to all parts of the dough.

5. The mechanical method indicates fermentation tolerance indirectly as far as this is related to the quality of the gluten ma-

terial. Flours that give good results by this method have good fermentation tolerance. Those that give poor results have to be somewhat coddled in the fermentation.

6. The mechanical method is more a test of protein quality than of quantity. For this reason the results are sometimes quite divergent from those obtained by conventional methods.

Literature Cited

Salmon, S. C., Swanson C. O., and Laude, H. H.
　　1927　Blackhull wheat in Kansas. Kans. Agr. Expt. Station. Bull. 241.
Swanson, C. O., and Working, E. B.
　　1926　Mechanical modification of dough to make it possible to bake bread with only the fermentation in the pan. Cereal Chem. 3:65-83.

EFFECT OF STAGE OF MATURITY ON COMPOSITION AND BAKING QUALITY OF MARQUIS WHEAT

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(Read at the Convention June, 1928)

New conditions and different methods of harvesting have been responsible for a renewal of interest in the relation of stage of maturity to yield and quality of wheat. The study described was undertaken primarily to answer some specific agronomic questions, but also to obtain data on the effect of maturity on quality and composition under North Dakota climatic conditions.

A complete review of the literature on this subject will not be attempted, but some of the more important work in this country and England will be cited briefly.

Humphries and Biffen (1907) discuss the relation of stage of maturity to baking quality. Grain cut at three different stages of maturity (green, ripe, and dead ripe) showed no significant or consistent difference in baking strength.

Brenchley and Hall (1909) harvested wheat at 3-day intervals from just after flowering until ripe. The total nitrogen content of the dry matter decreased rapidly at first but after the first six 3-day periods, remained fairly constant, and showed some indication of an increase as maturity was reached. They found that the actual weight of nitrogen in 1000 kernels tends to continue to increase until maturity is reached, and the relative ratio of starch

and protein deposition in the endosperm was fairly constant for different periods.

Kedzie (1893) of the Michigan station, reported analyses of wheat harvested at 24-hour intervals over a period of 46 days. The protein content of the dry matter decreased at first, but after dough stage remained fairly constant until mature.

Teller (1898), of the Arkansas station, reported analyses of wheat at different stages of maturity. Teller found that the relative proportion of gliadin tended to increase as the wheat matured, but glutenin tended to decrease slightly. Per cent of total protein in dry matter decreased from earliest cutting until about one week before normal harvest, when a tendency to increase was noted. Teller suggested that variation in seasonal climatic conditions may account for variation in results of different investigators.

Thatcher (1913), of the Washington station, reported protein content of Turkey and Bluestem wheat cut at different stages of maturity. The protein content showed a decrease followed by an increase as maturity is reached.

Shaw (1907) found that time of cutting after wheat reached hard dough stage has little, if any, influence on protein content.

Woodman and Engeldow (1924) found that the percentage of protein tended to increase as the kernel approached ripeness. The non-protein nitrogen and amino-acid nitrogen decreased as the grain reached maturity.

Stoa (1924), of the North Dakota station, found a slight but not significant difference in protein content and baking quality of Marquis wheat harvested early and late.

Experimental

The wheat used for this work was in all cases grown on fields of the Department of Agronomy, of the North Dakota Agricultural Experiment Station, at Fargo. The soil upon which this wheat was produced was the heavy clay type found in the Red River Valley. The fields used were probably in a relatively higher state of fertility than the average farm.

Marquis wheat was harvested at five different stages of maturity during the four years, 1924-1927. An extra late stage was added in 1927. Seasonal conditions vary from year to year, and the different plots were harvested when, in the opinion of the agronomist, the different stages of maturity were reached. It is

recognized that the stages considered are not sharply defined, and are determined by and dependent on the judgment of the individual.

The first grain was cut when the kernel reached the "dough" stage. During the four years the dough stage was reached on the average about 23 days after heading; the least number of days from heading to dough stage harvest was 22 and the greatest 24.

The second harvesting was when the grain reached the "hard dough" stage. This was usually 3 or 4 days after the first harvest. What was considered the "glazed" stage was reached 3 or 4 days later than the hard dough stage.

The normal harvest stage was considered as the stage when the grain was sufficiently firm that a reasonable amount of pressure by the thumb nail was necessary to make a dent in the kernel. In this region Marquis wheat requires about 35 days after heading to mature, or approximately 12 days after the "dough" stage is reached. In 1924, a cool year, the normal harvest stage was reached 14 days after the dough stage harvest. In 1925, the crop matured in 11 days after the dough stage harvest. In 1926, a dry and warm season, the crop matured in 10 days after, and in 1927, 12 days after dough stage. (See Table II).

From 4 to 5 days after reaching normal maturity the grain was considered dead ripe. For the extra late stage in 1927 it was allowed to stand 6 days after the dead ripe grain was harvested.

The wheat harvested at different stages was shocked and later threshed. Examinations reported were made on dry threshed grain.

In Table I are presented the average yields of grain obtained during the four years and the average weight per bushel of the wheat produced. The highest average yield, 26.3 bushels, was obtained from the plots harvested when the crop was considered normally ripe. The dead ripe and glazed stages were next highest. The lowest yield was obtained when wheat was harvested in the dough stage.

TABLE I
AVERAGE YIELD PER ACRE OF MARQUIS WHEAT AND AVERAGE WEIGHT PER BUSHEL AT DIFFERENT
STAGES OF MATURITY
Agricultural Experiment Station, Fargo, 1924 to 1927*

Plot No.	Stage of maturity when harvested	Average yield per acre, bu.	Average weight per bushel, lb.
1	Dough	20.7	52.3
2	Hard dough	23.2	54.2
3	Glazed	25.0	55.1
4	Normal ripe	26.3	55.1
5	Dead ripe	25.5	55.0

*In 1923 Marquis wheat harvested in the "normal" stage averaged 15.5 bushels, test weight 52.1 pounds, and that harvested 5 days earlier, 14.0 bushels per acre, test weight 51.0 pounds.

Stem rust reduced yields, particularly in 1925 and to some extent in 1927. High temperatures also affected yields in 1925, while temperatures in 1927, in spite of heavy rust infection, were particularly favorable to the filling of the grain.

The highest average weight per bushel of wheat was obtained from glazed, normal ripe, and dead ripe stages, there being no significant difference in test weight of the wheat cut at these stages. The dough and hard dough stages showed a lower average test weight.

Percentage of Protein in Wheat

The protein content of wheat harvested at different stages of maturity is shown in Table II and Chart 1. It will be noted that there is no consistent variation of protein content at different stages of maturity for the four seasons. In 1924 the protein content decreased from the dough to the glazed stage, but remained constant thereafter. In 1925 and 1926, protein content showed no significant or consistent variation. In 1927 protein content showed a marked increase between the glazed and the normal ripe stages.

TABLE II

EFFECT OF STAGE OF MATURITY ON PROTEIN CONTENT OF WHEAT
Marquis Wheat Grown at Fargo*

Stage of maturity	1924		1925		1926		1927	
	Date harvested	Per cent protein	Date harvested	Per cent protein	Date harvested	Per cent protein	Date harvested	Per cent protein
Dough	July 30	12.18	Aug. 1	12.97	July 26	12.30	Aug. 3	11.82
Hard dough	Aug. 2	12.34	Aug. 5	12.71	July 30†	12.47	Aug. 6	11.72
Glazed	Aug. 7	11.19	Aug. 8	12.95	July 31	12.33	Aug. 9	11.63
Normal ripe	Aug. 13	11.31	Aug. 12	12.78	Aug. 5	12.26	Aug. 15	12.22
Dead ripe	Aug. 18	11.39	Aug. 15	12.45	Aug. 10	12.60	Aug. 19	12.20
Extra late	....	....	....	....	....	....	Aug. 25	12.13

*Protein percentages calculated to 13.5% moisture basis.
†Would have been harvested July 29, but for rain.

Previous investigations show a lack of consistency in variations of protein content at different stages of maturity. Practically all previous investigations cover only one year's crop, but Teller suggests the possibility of climatic influence. Weather Bureau records for the four years, 1924 to 1927, show that differences in temperature and rainfall will account to some extent for the yearly variation shown in the chart.

In 1924, during the maturing period, the weather was unusually cool and 1.34 inches of rainfall is recorded between the hard dough and the glazed stages. This probably accounts for the decrease in percentage of protein during that season. In 1927, an increase of protein occured between the glazed and the normal ripe

stages. Weather Bureau records show a sharp increase in temperature for this period and practically no rainfall. After grain reaches the normal ripe stage, the protein content apparently remains practically constant.
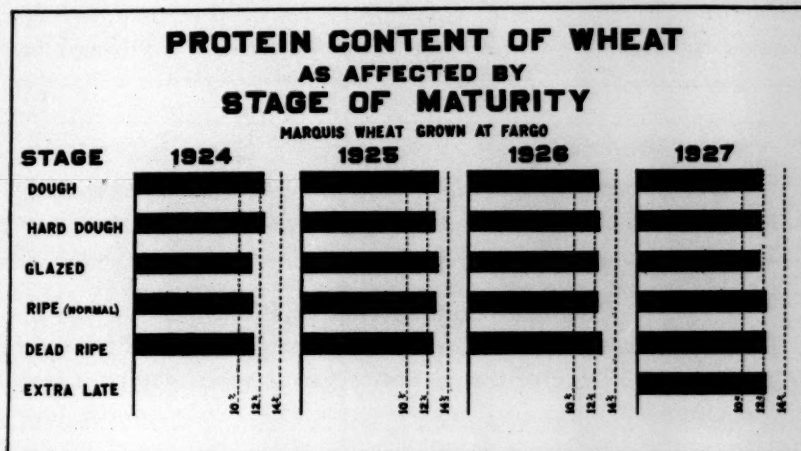
Baking Quality of Flour

Samples of the wheats harvested at different stages of maturity were milled and baked. In the 1924, 1925, and 1926 crops, a straight-grade flour was prepared, but in the 1927 crop samples, a 75 per cent patent flour was milled.

The baking tests with straight-grade flours for the three years showed a tendency for the quality of the flour to improve as the wheat became more mature. The loaf volume, color, and texture of bread from wheat in the normal ripe and dead ripe stages averaged higher than of bread from wheat in the less mature stages. A tendency to poor color in bread from wheat in the dough stage was noted in 1925 and 1926.

For baking tests with straight-grade flours, 340 grams of flour were used; with patent flours from the 1927 crop, 100-gram samples were used. The results of the baking tests on 1927 samples are given in Table III. There is evidently little difference in baking quality of the patent flours milled from Marquis wheat harvested at six different stages of maturity. There is no significant change in loaf volume, as the maximum variation is only 7 cc. The color

scores were the same for all loaves, and in these patent flours did not show the poor color in the dough stage.

The texture scores, however, do show a difference in favor of the more mature samples; those for the dough and hard dough stages are lower.

TABLE III

BAKING DATA ON 75% PATENT FLOURS FROM MARQUIS WHEAT HARVESTED AT DIFFERENT STAGES OF MATURITY*

Stage of maturity	Loaf volume cc.	Color score	Texture score
Dough	383	94	93
Hard dough	378	94	93.5
Glazed	378	94	94.5
Normal ripe	383	94	94
Dead ripe	385	94	95
Extra late	380	94	94.5

*Grown at Fargo, 1927 Crop.

Chemical Composition of Flour

The chemical examination of the straight-grade flours from 1924, 1925, and 1926 crops was limited to protein determinations. A more extensive chemical examination was made on the patent flours from the 1927 crop samples. The chemical examination of these samples covered essentially protein distribution, sugar content, and diastatic activity.

Table IV shows protein, ash, and sugar content and diastatic activity of patent flour from wheats harvested at different stages of maturity in 1927. The protein content of the flour, as might be expected, shows variation similar to the protein in wheat (Table II). The ash content tends to decrease as the wheat becomes more mature. This indicates that the more mature wheat is more satisfactory for milling. The sugar content of these flours is remarkably constant for the different stages of maturity. The ratio of sugar to other constituents, therefore, appears to be normal when the dough stage is reached.

TABLE IV

COMPOSITION OF PATENT FLOUR FROM MARQUIS WHEAT HARVESTED AT DIFFERENT STAGES OF MATURITY*

Chemical Data on 15% Moisture Basis

Stage of maturity	Per cent protein (Nx5.7)	Per cent ash	Per cent total sugar calc. as invert sugar	Per cent reducing sugar	Diastatic activity mg. maltose per 10 gm. flour
Dough	10.69	0.494	1.80	0.09	77.9
Hard dough	10.74	.493	1.80	.12	72.8
Glazed	10.62	.436	1.79	.15	52.9
Normal ripe	11.02	.436	1.84	.14	68.4
Dead ripe	10.96	.420	1.75	.09	61.7
Extra late	10.94	0.424	1.72	0.13	56.7

*Grown at Fargo, season of 1927.

The diastatic activity of the patent flours from wheat at different stages of maturity is shown in Table IV. Diastatic activity was determined by Rumsey's method. There is a tendency for the diastatic activity to decrease as the grain matures, but the decrease is not consistent from stage to stage. The highest diastatic activity was found in the dough stage and the lowest in the extra late stage.

Nitrogen or Protein Distribution at Different Stages of Maturity

It is apparent that the total nitrogen or protein content does not show much change from the dough to the mature stage (Tables II and IV). To determine the relative proportions of different types of protein or nitrogenous material at different stages of maturity, the following determinations were made. Nitrogen soluble in 70 per cent alcohol, and nitrogen soluble in 5 per cent potassium sulphate solution were determined. A measure of amino acids present was secured by Sørensen's titration method and by determining the amount of nitrogen not precipitated by tungstic acid. Glutenin was determined by the barium hydroxide method of Blish.

The data for these determinations on patent flours are given in Tables V and VI. With the exception of the hard dough stage, the ratio of glutenin to total protein is remarkably constant for the first four stages. The proportion of glutenin tends to decrease in the dead ripe and extra late stages.

The nitrogen soluble in 70% alcohol (calculated as protein) shows a consistent increase from the dough to the extra late stage. The nitrogen soluble in 5% potassium sulphate solution (calculated as protein) shows a consistent decrease until the normal ripe stage is reached, after which it remains constant.

TABLE V

PROTEIN DISTRIBUTION IN PATENT FLOUR FROM MARQUIS WHEAT HARVESTED AT DIFFERENT STAGES OF MATURITY*

Stage of maturity	Protein in flour (Nx5.7)	Glutenin Ba(OH)₂ method		Protein soluble in 70% alcohol		Protein soluble in 5% K₂SO₄	
		Per cent in flour	Per cent of total protein	Per cent in flour	Per cent of total protein	Per cent in flour	Per cent of total protein
Dough	10.69	4.33	40.5	5.07	47.4	1.72	16.1
Hard dough	10.74	4.53	42.2	5.20	48.4	1.61	15.0
Glazed	10.62	4.30	40.5	5.33	50.1	1.51	14.2
Normal ripe	11.02	4.44	40.3	5.81	52.7	1.53	13.9
Dead ripe	10.96	4.34	39.6	5.88	53.6	1.53	14.0
Extra late	10.94	4.10	37.5	5.92	54.1	1.54	14.1

*Grown at Fargo, 1927 crop.

TABLE VI

AMINO ACID ON NON-PROTEIN NITROGEN IN PATENT FLOURS FROM MARQUIS WHEAT HARVESTED AT DIFFERENT STAGES OF MATURITY*

Stage of maturity	Per cent amino acid nitrogen Sørensen's formol titration	Per cent nitrogen not precipitated by tungstic acid
Dough	0.009	0.027
Hard dough	.008	.024
Glazed	.007	.020
Normal ripe	.008	.020
Dead ripe	.007	.020
Extra late	0.007	0.020

*Grown at Fargo, 1927 crop.

Amino nitrogen as determined by Sørensen's titration method does not show much variation for the different stages of maturity. The dough stage shows the highest percentage.

The nitrogen not precipitated by tungstic acid is highest in the dough stage and decreases until the glazed stage is reached. After that the percentage of nitrogen not precipitated by tungstic acid remains constant.

The principal change noted in the protein material of the patent flour at different stages of maturity is the tendency for the alcohol soluble material to increase as the potassium sulphate soluble material decreases. The glutenin is fairly constant, but shows a tendency to decrease in relative proportion to total protein at latest stages.

Discussion

From an economic standpoint it is not profitable to harvest wheat before normal ripe stage is reached. There is apparently no serious loss in yield if wheat is allowed to stand a reasonable length of time after this stage is reached.

From the standpoint of baking quality and chemical composition there is less difference between mature and immature stages than might be expected. The data indicate that the dough and hard dough stages are probably less stable in baking quality, but the difference is not great.

In chemical composition the most significant change was the consistent increase of protein soluble in 70% alcohol, and the tendency for diastatic activity to decrease. The flour from dough and hard dough stages contained slightiy more non-protein nitrogen, and a greater proportion of protein was soluble in 5% K_2SO_4 than in more mature stages.

The data indicate that the composition of the wheat kernel remains fairly constant after the dough stage is reached. Data from

some of the early investigations indicate considerable variation in composition previous to the dough stage. Insufficient study of the results of these earlier investigations with reference to stage of maturity is probably responsible for the rather common impression that wheat decreases consistently in percentage of nitrogen or protein as it reaches maturity. The percentage of protein evidently does show a sharp decrease with maturity until the dough stage is reached. After the dough stage is reached, however, the percentage of protein remains fairly constant, and may show slight increases or decreases, depending on prevailing weather conditions.

Summary

1. Marquis wheat harvested in the dough and hard dough stages averaged lower in yield and test weight per bushel than that cut at a more mature stage. There was no increase in yield after the crop reached normal maturity.

2. Protein content of wheat showed no consistent variation at different stages of maturity for different seasons. Variation in prevailing climatic conditions during maturing period is probably responsible for lack of consistency in protein content variation for different stages of maturity.

3. The protein content of patent flour from 1927 crop samples varied as did protein in wheat. Ash decreased as wheat matured, but sugar content remained constant from dough to extra late stage. Diastatic activity showed a tendency to decrease as wheat matured.

4. Baking tests with straight-grade flours from wheat harvested at different stages of maturity showed slighly better quality for the mature wheats. Baking tests with patent flours from wheat of the 1927 crop, harvested at different stages of maturity, show no significant difference in loaf volume or color, but those from the dough and hard dough stages were lower in texture.

5. Nitrogen distribution in flour from wheat harvested at different stages of maturity showed some variation, but less than would be expected. Glutenin remained practically constant, but tended to decrease in dead ripe and extra late stages. Nitrogen soluble in 70% alcohol showed consistent increase with maturity, while nitrogen soluble in 5% K_2SO_4 showed consistent decrease.

Nitrogen not precipitated by tungstic acid and amino nitrogen was highest in the dough stage, but was at practically a constant level after the glazed stage.

Literature Cited

Brenchley, W. E., and Hall, A. D.
  1909 The development of the grain of wheat. J. Agr. Sci. 3:195-217.
Humphries, A. E., and Biffen, R. H.
  1907 The improvement of English wheat. J. Agr. Sci. 2:1-17.
Kedzie, R. E.
  1893 Composition of wheat at different periods of ripening. Mich. Agr.
        Expt. Sta. Bull. 101.
Teller, G. L.
  1898 A report of progress in investigations of chemistry of wheat.
        Ark. Agr. Expt. Sta. Bull. 53.
Thatcher, R. W.
  1913 The progressive development of the wheat kernel. J. Am. Soc.
        Agron. 5:203-213.
Shaw, G. W.
  1907 Report of progress in cereal investigations. Calif. Agr. Expt. Sta.
        Bull. 185.
Stoa, T. E.
  1924 Early harvest of rusted Marquis wheat. J. Am. Soc. Agron. 16:41-46.
Woodman, H. E., and Engeldow, F. L.
  1924 A chemical study of the development of the wheat grain. J. Agr.
        Sci. 14:563-586.

FACTORS INFLUENCING CHECKING IN BISCUITS[1]

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Introduction

The phenomenon with which this paper has to deal is widespread, involving many industrial processes other than biscuit baking. In the manufacture of alimentary pastes, for example, we are impressed with the skillful technic that has been developed in an effort to control checking. In other industrial fields, more or less elaborate procedure has been adopted either as the result of generations of experience on the part of the artisan or as the direct result of scientific research, in the hope that checking may be entirely eliminated, or its effect minimized.

Doubtless everyone is familiar with the checking of wood. A splitting, or the development of a small crack, usually in the central portion of the article, is a familiar sight to those who have occasion to examine old cabinet work, musical instruments, or furniture. If checking is unwelcome and annoying to the violin maker, it is equally so to the biscuit baker; for checked biscuit should not be packed with perfect biscuit—they are almost certain to be broken before reaching the consumer. In most cases, however, the biscuit are packed before the checking has proceeded so far as to be visible and the consumer is likely to find a considerable portion of the contents of the carton either entirely broken or so badly checked as to break with the slightest jar; in which case the reputation of the biscuit manufacturer suffers. The control of checking is a problem of no small importance, as considerable sums of money are annually lost through its existence.

The occurrence of checking in the commercial biscuit plant and the consequent effort to control conditions with a view to eliminating it present a problem replete with theoretic interest and at the same time intensely practical. For many years the biscuit baker has been troubled with the appearance of small cracks in the central portion of the biscuit. These frequently extend so close

to the periphery that the whole structure is weakened and the slightest jar may break the biscuit into two or more fragments. These small cracks are often observed in English type hard-sweet biscuit, which seem to be most easily affected. Soft-dough types are seldom affected; nor is checking important in the manufacture of sponge goods, such as soda crackers. The study of checking is best undertaken in the hard-sweet types, and our investigation was limited largely to varieties of biscuit that fall within that class.

Figure 1 illustrates some English hard-sweet biscuit in a badly checked condition. Unless handled very carefully, biscuit in such condition will easily break. All the specimens shown are unsalable.

Fig. 1. Hard-Sweet Biscuit, Showing "Checked" Condition

Altho many attempts have been made to deal with the subject in a practical way, as evidenced by numerous references in the trade press devoted to biscuit problems, few references to it are found in scientific literature, and it is fair to assume that little attempt has been made to apply scientific methods in an endeavor to eliminate checking. In a practical way, every ingredient of the dough mixture has been closely scrutinized. There appears to be

a tendency on the part of the practical biscuit baker to believe that there is one cause of checking, rather than to assume that it may be the result of a series or combination of conditions. One group of bakers assumes that the type of flour used is the all-important factor. Another group has suggested that the character of the leavening agent is important. Still others believe that the method of mixing the dough is of no small consequence, while there seems to be a tendency to scrutinize the baking and curing processes, hoping to discover which conditions are most likely to eliminate checking.

In our consideration of the problem of checking, its causes, and control measures that may assist the baker in its elimination, our procedure was directed in the following manner. After a brief resumé of the general subject of checking, which included an investigation of the various methods of control used, not only in the biscuit industry but in other fields as well, we set up a hypothesis as to the reasons for its occurrence. This was of necessity theoretical in nature. Having established a working hypothesis, we set about testing each assumption, by either our own experimental work or that of others. After having justified our hypothesis and shown its possibilities, we endeavored to produce checking experimentally by setting up conditions which, according to our hypothesis, should be ideal for the occurrence of checking. Having demonstrated our ability to produce a checked biscuit at will, we investigated the effect of several methods of prevention that have been advanced by bakers, attempting to show whether or not they were in accord with our hypothesis.

The dough from which hard-sweet biscuit are manufactured is very complex, consisting principally of flour, fat, water, sugar, eggs, flavoring, and a chemical leavening agent. Sometimes a small amount of starch, such as arrowroot starch, is added. If the dough is mixed carefully we can safely assume that the structure is fairly uniform throughout. The system is somewhat like that described by Ostwald (1919) for bread dough.

A biscuit dough is a polydispersoid. Water is the dispersion medium; and uniformly distributed therein are molecularly dispersed particles, colloidally dispersed particles, and coarsely dispersed particles. Under "molecularly dispersed particles" are sugars, chemical leavening agents (sodium or ammonium bicarbonate), and common salt ($NaCl$). Under the same head we may also classify such dissolved gases as carbon dioxide, or oxygen. The dissolved

proteins and starch are colloidally dispersed, whereas the undissolved or partly dissolved starch grains, gas bubbles under certain conditions, and fat globules are in the coarsely dispersed state.

The most important ingredient of the dough mixture is the flour. Ostwald (1919) described flour as a coarse dispersion of several poor-in-water hydrogels, which he classifies as follows: First, and perhaps the most important constituent, is the protein gel, mechanically disintegrated and without special structure. Second, the starch granules and the cellulose gel derived from the cell walls, both of carbohydrate nature. Until recently the latter class has been looked upon as more or less inert material, making up the bulk of the dough. However, there is evidence, according to Alsberg (1927), of some variation in the properties of starches. Finally, the individual gel particles contain in molecular dispersion such substances as sugars, organic acids, inorganic salts, and water. Gases are present in two states—a portion adsorbed upon the surface of the particles and the rest in true solution in the water.

Collectively, dry flour may be regarded as a coarsely dispersed gel powder. Each gel particle may contain molecularly dispersed constituents, such as salts, sugars, acids, and especially water. Gases are not only in solution, but are often either adsorbed upon the surface of the gel particle, or occluded within it. Air, or more properly a mixture of air and carbon dioxide, is the connecting phase or dispersion medium. Flour must be considered a gel powder, as the particles are practically all more than two-tenths of a micron in diameter and are therefore microscopic. Altho the smallest flour particle is visible under the microscope, it is fitting that flour be considered as a colloid, as each flour particle is made up of primary units that are of colloidal dimensions.

Another important ingredient of the dough mixture is water. Fundamentally, the simplest dough is a mixture of flour and water. This is of interest historically, for there is little doubt that the bread of primitive man was baked from such dough. While the regulation of the amount of water added to the flour was of small importance to the primitive man, who baked his bread on a flat stone and gave little attention to symmetry of loaf, the determination of the proper absorption for a given flour is of the utmost importance in modern baking practice. The bread baker has paid much attention to the question of proper absorption, which is usually determined by the "feel" of the dough. Harrel (1927)

recently devised a penetrometer, which is of considerable assistance to a baker in maintaining uniformity in dough from day to day. It should also be of no little assistance to the biscuit baker, for the regulation of absorption is of considerable consequence, particularly with doughs run on a pan-and-scrap machine.

As the simplest dough is formed by mixing flour and water, all other ingredients may be considered as factors influencing the physical properties of this fundamental dough. The influence of the various ingredients usually found in biscuit doughs can be best ascertained by a survey of the common types of commercial biscuit. The simplest commercial type of biscuit is hard-tack, which is made by mixing flour, salt, and water. The biscuit resulting from such a dough is hard and quite coarse, the inside consisting of comparatively few thick layers. The principal advantages of such a biscuit are that it is cheap and that it will keep for many months under almost any climatic conditions. If a small amount of shortening (about 15 pounds per barrel) is added to the hard-tack dough, the resulting product is commonly called pilot bread. Owing to the action of the shortening, the resulting product is less hard and considerably more tender than hard-tack. The biscuit is crisper and has a much more pleasing color and flavor. In keeping qualities, pilot bread, however, is not equal to hard-tack, owing to the possibility of the fat becoming rancid. If the amount of fat is increased, the flakiness and shortness of the finished product are increased until the product finally obtained is not unlike pie crust.

Sugar should never be added to the simple flour and water dough without an accompanying addition of shortening or the dough will be sticky and the resulting product hard and unappetizing. When a small amount of sugar (approximately 40 pounds per barrel) is used, with shortening (about 20 pounds per barrel), the resulting product is usually termed a hard-sweet. This class includes wine biscuit, arrowroot biscuit, and social tea biscuit. The dough for these is mixed stiff. It is tough and not unlike hard-tack dough in appearance. The hard-sweet biscuit, however, are fairly crisp and tender. The texture is much finer than that of hard-tack and the biscuit takes on a rich, yellowish brown on baking.

If the amount of sugar and shortening is increased to approximately 75 pounds and 30 pounds, respectively, per barrel, the product resulting is termed a sugar cookie. If we increase the content

of sugar to 100 or 125 pounds per barrel, at the same time keeping the shortening content at about 30 pounds, as before, the product is usually referred to as a snap—lemon snaps or ginger snaps. If, on the other hand, the sugar content is held at approximately 75 pounds per barrel, as in the sugar cookie, and the shortening content is increased, the resulting biscuit is called Scotch shortbread. This type of biscuit is close in texture, very tender, and may be readily recognized by the tendency toward greasiness in the finished product.

Shortening makes the dough more friable and less sticky. From the standpoint of commercial production of biscuit, the latter consideration is of considerable importance. A dough that is too sticky will stick to the rolls and the cutter to such an extent that it is practically impossible to handle it in a commercial biscuit machine.

The outstanding property of the fat, or shortening ingredient, from the standpoint of checking control, is that it is completely insoluble in the dough mixture. All other important ingredients used in the dough are either soluble in or miscible with water. Flour, for example, contains two principal proteins—gliadin and glutenin—both of which, altho fairly insoluble in water, imbibe water freely during the mixing process, producing a sticky mass usually referred to as gluten.

Several explanations have been offered as to the mechanism of the mixing process. Most of these explanations, however, are largely hypothetical, having few experimental data to substantiate them. There is little doubt that the chief end of the mixing process is a thoro and uniform hydration of the dry materials in the dough. In a biscuit dough, the supply of water is limited, and doutless several substances are competing for a share. (1) There is usually an excess of sugar, at least in most doughs, and a considerable portion undoubtedly tends to dissolve in the water added. (2) The gluten proteins have a large imbibitional capacity, and this portion of the dough mixture probably imbibes a considerable portion of the moisture. While it is true that raw starch does not ordinarily imbibe much water, Alsberg (1927) has shown that cooked or gelatinized starch has a high imbibitional capacity. The effect of the added salt and the leavening agents is probably larger than might appear to be the case on first thought, as Sharp and Gortner (1923) have shown that the imbibitional properties of

proteins may be considerably influenced by the amount and character of the electrolytes in the system.

If shortening is incorporated in a dough, however, the small particles that make up the dough become partially coated with fat so that they do not coalesce and stick together so easily when brought in contact with one another by the action of the mixing machine as they might if no shortening is present. The result is that the thin layer of shortening between the dough particles tends to prevent close coalescence, and the resulting product is much more friable and considerably more appetizing. These thin layers of shortening in the dough break up the continuity of what would otherwise be a more or less solid and continuous mass.

We have already indicated that the outstanding physical property of shortening, which distinguishes it from all other ingredients of the dough, is its complete insolubility and immiscibility with water. If we conceive of a dough as being made up of small hydrated particles more or less completely surrounded with a thin film of shortening, then Ostwald's conception of a bread dough does not apply to biscuit doughs, in which the shortening is in considerably larger amounts. From Ostwald's point of view, a bread dough is a polydispersoid in which water is the continuous phase. In a biscuit dough the disperse phase is the hydrated dough particles, the shortening acting as the dispersion medium. Such a concept may not hold strictly in biscuit doughs of small shortening content, but as the shortening content is increased there is more likelihood that the dough structure will resemble the system described.

When we consider the baked biscuit in the light of this new conception of dough structure, it appears that shortening might exert two important influences, which bear directly on the problem of checking. In the first place, the movement, or migration, of moisture in the various portions of the biscuit will undoubtedly be materially retarded. Evaporation from the surface of the freshly baked biscuit will not take place as rapidly in biscuit with a high shortening content as in those in which the shortening is in only small amounts. This effect of shortening will be referred to later. The second effect of shortening, according to our new conception, is more or less closely associated with the theory of the action of shortening as advanced by Platt and Fleming (1923). As shortening is present in innumerable minute cracks or breaches of continuity of the hydrophyllic materials throughout the structure of the biscuit, there will be a certain ductility in the structure.

If strains are set up in certain portions of the biscuit, which is likely to occur, as will appear later, a movement may result that will tend to compensate for such strains. The biscuit structure might then appear to be capable of slight stretching or compression, during which process the fat, which fills the interstices between the hydrated dough particles, may flow slightly, allowing the dough particles to move either farther apart or closer together and thus relieve the strained condition. If such a conception is correct, it appears likely that checking should occur in biscuit that contain no shortening or only relatively small amounts, while biscuit that contain a considerable amount of shortening should have little tendency to check. Practical experience has shown that such is the case, for Scotch shortbreads seldom check to any great extent whereas hard-sweets and other types of biscuits that are low in shortening are most susceptible to checking.

Until a comparatively few years ago, little was known with regard to the action of shortening in baked goods. During recent years shortening manufacturers and cereal chemists have turned their attention to this, the most important problem of the biscuit baker. Platt (1922) has thrown considerable light on the action of shortening in baked goods, and Platt and Fleming (1923) applied theoretical considerations such as were outlined by Harkins and his co-workers (1917, 1920, 1921), and Langmuir (1917). Their deductions from the work of Harkins and Langmuir indicate that one of the important factors regarding the shortening power of any fat is its content of unsaturated glycerides, the shortening value varying directly with the unsaturated glyceride content. From theoretical considerations, Platt and Fleming point out that the presence of unsaturated structures in the fat molecule increases the tendency of the shortening to adhere more closely to the surface of the hydrated dough particles, so more pressure is required for two layers of dough to break through the intervening layer of the shortening; thus forming a weaker union between the dough particles.

It is easy to see from the foregoing brief discussion, that many factors are to be considered in choosing a shortening. We have not attempted to indicate all the important shortening problems, but have mentioned one or two that may have a bearing on the present problem. The requirements that a shortening must meet vary considerably with the type of baked goods, as conditioned by price, availability, and convenience.

Sugar is another important ingredient of a biscuit dough. Cane or beet sugar, that is, sucrose, affects the properties of the dough, making it much softer and stickier. The dough seems much shorter, and curiously enough, much less water can be used in making it. For example, hard-sweets, which contain about 15 units of sugar per 100 units of flour, require 50 per cent more water than sugar cookies containing 37 units of sugar, and the latter dough is softer and stickier than the hard-sweet dough. That the water requirement varies inversely with the shortening content will be demonstrated later. The two doughs just referred to contain approximately the same amount of shortening, so the effect of shortening on absorption does not enter into the discussion.

Altho sucrose tends to make the dough short, it also tends to make the biscuit hard and tough unless sufficient shortening is used to counteract this tendency. Sugar also tends to make a biscuit flatten out or "spread" during baking, often to such an extent that the diameter of the finished biscuit exceeds the diameter of the dough disc by 25 per cent.

Without sugar, biscuit puff up excessively, giving rise to a product that is too open in texture. Such goods are said to be "poor." If sufficient sugar is incorporated in the dough, the resulting biscuit will be close in texture and of pleasing color and contour. Such goods are said to be "rich." These trade terms are also used to denote the lack or sufficiency of shortening. While the two meanings of these terms are distinct, their indiscriminate use often leads to confusion.

Honey, invert sugar syrup, and dextrose do not produce results in biscuit similar to those produced by sucrose. Whereas biscuit made with sucrose tend to be hard and flat, those made with invert sugar tend to be soft and spongy. The peculiar grain imparted by invert sugar is characteristic and cannot be obtained in any other way. The hygroscopicity of these sugars tends to keep the biscuit soft. This is a very desirable quality, as will be brought out later. Invert sugar caramelizes at a lower temperature than sucrose, and consequently biscuit containing invert sugar will color more readily than those made with sucrose.

Several types of leavening agents are used in biscuit manufacture. Among the more important are ammonium bicarbonate, tartaric acid, calcium acid phosphate, and sodium bicarbonate. Ammonium bicarbonate is an ideal leavener for hard cakes because

it is completely volatile and gives rise to more gases per unit of weight than any other leavener in common use. The leavener opens the structure of the biscuit, making it more tender and friable. In this, leaveners act in some measure like the shortening ingredients. In fact, excessive amounts of leaveners are sometimes used in goods that are poor in shortening to compensate for the toughness due to the small amount of fat. When acid ingredients are used with sodium bicarbonate, the action is that of a common baking powder. When molasses is used, the acid of the molasses acts on the soda in the same manner. In biscuit manufacture, however, soda is usually used in excess; in fact, soda and ammonium bicarbonate are often used together without any acid ingredient. The effect of the alkalinity imparted by the excess soda is to hydrate the gluten, increasing the spreading of the piece and improving the smoothness of the bottom, at the same time accelerating the browning. Without an excess of soda, it is practically impossible to brown a piece evenly if made with sucrose unless a large amount of fat is used in the dough mixture. A high heat or a long bake, in the absence of excess shortening or soda, only tends to burn the edges without browning the top properly.

The foregoing discussion covers the action of the principal ingredients of sweet biscuit. Almost an infinite variety of ingredients may be added to a biscuit dough, such as flavoring, coloring material, eggs, fruit, and nuts. It seems inadvisable to discuss the action of such materials as they play but a small rôle in checking.

The Hypothesis

The complex mixture of flour, fat, water, sugar, and other ingredients, which makes up biscuit dough, enters the oven in a moist condition. The moisture content may vary from 10 to 40% in sweet biscuit, depending on the type of flour used and the relative proportion of sugar and shortening. This wet material undergoes several important changes during the process of baking. In the first place, the gluten of the flour is coagulated by the heat and converted from an elastic substance into one more or less rigid. This change may be compared with the changes in egg white when it is coagulated by heat, altho the analogy is not strictly identical. Alsberg and Griffing (1927) have followed the action of heat on gluten. Their work will be referred to later. During this process, the gluten loses its capacity to hold water. Starch, however, increases in capacity to take up water during the

later stages of baking. Raw starch will absorb comparatively little water; cooked starch is gelatinized and takes on water, assuming a jelly-like consistency.

Both the coagulated gluten and the gelatinized starch lose water as the baking process is continued. The unfortunate feature of the situation is that all parts of the piece do not lose water at the same rate. Altho a thoroly baked biscuit may appear dry, it contains appreciable quantities of water when it leaves the oven. It seemed reasonable to assume that the central and interior parts of freshly baked biscuit might contain considerably more water than the outer parts and the rim of the biscuit, because the outer rim of the average biscuit is somewhat browner than the central portion.

Immediately as the biscuit leaves the oven, it is probable that a contest for the water present ensues. The drier parts along the rim, or periphery, tend to take up water from the moister regions near the center as well as from the atmosphere. As the dry portions absorb water, they increase in size. This phenomenon is widespread in nature and is typical of such plant fiber gels as wood, cotton, and linen, and of most animal structures. Conversely, the moist portion, that is, the central portion of the biscuit, should tend to shrink in size because it is losing part of the water it contained when removed from the oven. If this difference in moisture between the interior and the exterior portions of the biscuit is of considerable magnitude and the biscuit is cooled rapidly, conditions appear ideal for checking to occur. Experience has taught us that the biscuit is not rigid when freshly baked and still warm, even tho it may be baked to a fairly small moisture content. This is because the gel structure has not "set." In cookies, which contain a fairly large amount of sugar, it is likely that the stiffening, which takes place upon cooling, is analagous to the stiffening of taffy or other hard candy when the concentrated sugar solution is cooled to ordinary temperatures. Bingham and Jackson (1917) studied the physical properties of sugar solutions. While their experimental data do not extend into the range of concentration or temperature with which we are concerned, their findings indicate that the sugar solution plays a major rôle in the plastic nature of the biscuit while warm, and in the hardening that occurs upon cooling. When the average biscuit is removed from the oven it can be easily bent around the finger, and if held in that position until cool it is impossible to straighten it again,

hence it seems unlikely that checking should originate while the biscuit is pliable.

Once the cookie has cooled to approximately room temperature and the gel has "set" to a more or less rigid form, conditions are no longer the same. Any strains that might arise later would be opposed by the elastic properties of the rigid biscuit structure.

It is easy to see why strains should be set up in the biscuit at this point and subsequently. If the internal portion of the biscuit is losing moisture and tending to shrink while the external portion, or the periphery, is taking up moisture and tending to expand, we have the two portions of the biscuit attempting to move in opposite directions. If the forces developed are of sufficient magnitude to exceed the tensile strength of that portion of the biscuit structure which is under strain, checking should be inevitable. The portion of the biscuit in which the strain should be greatest is in the region that lies half way between the external, or expanding, section and the internal, or contracting, section. That checking actually does manifest itself in this region will be brought out later.

At the outset, it seems likely that checking would depend upon the spread in moisture content between the internal and the external portions of the freshly-baked biscuit; also that the relative humidity of the atmosphere might play an important rôle in the checking phenomenon. The worst conditions that might be imagined are those in which it would be necessary for the internal portion of the biscuit to lose moisture and the external portion to gain moisture in order to come into hygroscopic equilibrium with the relative humidity of the atmosphere. Ideal conditions for preventing checking would be those in which the moisture content of various portions of the biscuit is fairly uniform. It also seems advisable to keep the biscuit warm (and consequently plastic) as long as possible, for heating facilitates the transfer of moisture in gel structures, and the equalization of the moisture gradient of the biscuit would take place, in large measure, while the biscuit is still plastic. In such case, expansion or contraction might take place without causing the biscuit to check.

It also appears likely that biscuit with a fairly open texture, that is, a texture that is fairly large in volume per unit of weight, should have less tendency to check. Such biscuit should be more readily desiccated to the desired degree in the central portion than one of more dense nature. The placing of dockers, or small

metal stylusses (see Fig. 2) which produce the small holes in the central portion of the biscuit, should alleviate checking, as the holes assist in the removal of moisture from the interior of the biscuit during baking.
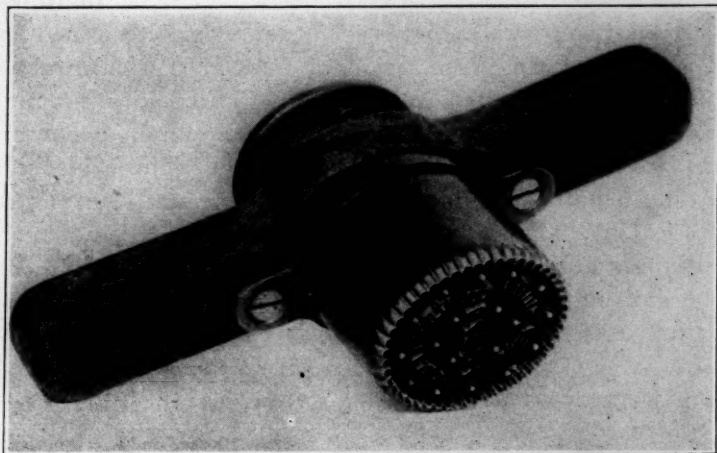

Fig. 2. Hand Biscuit Cutter Showing Dockers

At the outset, it appeared improbable that manipulation of the formula would control checking, but that conditions of baking and of subsequent curing of the biscuit would be a more fruitful field of investigation.

Experimental

The first series of experiments was undertaken to ascertain whether a spread in moisture or a moisture gradient exists in the freshly baked cookie, and to determine the magnitude of the spread under actual plant conditions. By spread in moisture is meant the difference in moisture content of the outer, drier portion of the biscuit and the inner portion, which is more moist. A special cutter was designed with which the freshly baked biscuit might be cut into a series of concentric rings or zones. This cutter is illustrated in Figure 3.
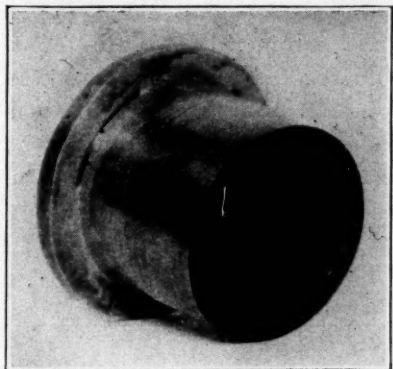
Fig. 3. Cutter Devised for Cutting Biscuit into 4 Zones or Areas

Four freshly baked biscuit were cut, each biscuit being divided into four zones and an outside rim, or periphery, making five fractions in all. Corresponding portions were sealed in glass-stoppered bottles, and the total weight and moisture content of each were determined. In this experiment and in all others referred to in this paper, moisture content was determined by the official method of the Association of Official Agricultural Chemists. The data are shown in Table I. The cookies examined in this experiment were taken from a regular run in a commercial biscuit plant under average plant conditions. These biscuit were of the type usually designated as honey cake and the dimensions were 73 × 73 × 9.5 mm. These biscuit were baked in a reel oven for 7 minutes at a temperature of 220° C.

The data in Table I show that there is an appreciable spread in moisture in the various sections of a commercially baked biscuit.

TABLE I

MOISTURE CONTENT OF VARIOUS PORTIONS OF FRESHLY BAKED HONEY CAKE

Zone	Moisture content %
Outer rim	7.6
Zone 1	11.7
Zone 2	12.6
Zone 3	13.4
Zone 4 (center)	14.2

It seemed advisable to repeat the foregoing experiment, this time with a biscuit that was thinner and of a harder type. The sample selected was of the hard-sweet variety, and was similar to those shown in Figure 1. The dimensions were 50 × 44 × 5 mm. Five biscuit were cut into concentric zones immediately after baking, and like zones were rapidly sealed. The samples were taken at random from a run in a commercial biscuit plant. The time of baking was 7 minutes and the oven temperature was 225° C. The data are shown in Table II. The experiment was carried out in duplicate. Samples for series 1 were taken at the beginning of the run, those for series 2 in the middle of the run.

TABLE II

MOISTURE CONTENT OF VARIOUS PORTIONS OF FRESHLY BAKED
HARD-SWEET COOKIES

	Series 1 (beginning)	Series 2 (middle)
	%	%
Zone 1 (rim)	0.54	1.65
Zone 2	1.58	2.08
Zone 3	3.45	3.66
Zone 4 (center)	5.05	3.93

It is evident that there is a considerable spread in moisture in freshly baked biscuit under commercial conditions.

Similar experiments were conducted with other types of biscuit, results being similar to those in Tables I and II.

Another series of experiments was undertaken to determine the rate at which the various zones either gained or lost moisture during the first few hours after the biscuit was removed from the oven. The biscuit selected for this series was of the hard-sweet type. It was a chocolate wafer designed for a sandwich with a cream filling. The dimensions of this wafer were 50 × 60 × 4 mm. Several cookies were zoned with the cutter shown in Figure 3, and the zones were analyzed for moisture content at various intervals subsequent to removal from the oven. These cookies were baked for 7½ minutes in a commercial reel oven at 215° C. The relative humidity in the bake shop was 24 per cent. Some of these cookies showed a tendency to check when left exposed for a few days in the shop. The data are shown in Table III.

TABLE III

RATE OF EQUALIZATION OF MOISTURE GRADIENT OF FRESHLY BAKED HARD-SWEET WAFER

Time, hr.	Moisture content, %			
	Zone 1 (rim)	Zone 2	Zone 3	Zone 4 (center)
0	3.53	5.57	6.67	6.73
¼	3.87	5.20	6.30	6.50
½	4.00	5.30	6.60	6.20
1	4.07	4.97	5.73	5.87
4	4.20	4.77	5.40	5.47
30	5.00	5.13	5.53	5.53

The data in Table III demonstrate that the exterior of the biscuit, designated as zone 1, took up moisture in order to come into equilibrium with the relative humidity of the atmosphere. The moisture content of zone 2 changed little, whereas the central zones both lost moisture.

Another series of experiments was undertaken to determine whether the shape of the biscuit was important. Three types of biscuit were being manufactured on the same day in the plant under observation, the dough formulas for each being identical. The shapes of these biscuit may be described as square, rectangular, and oval. All the samples were baked for 7 minutes at a temperature of 220° C. The relative humidity in the shop was 40% Samples were zoned immediately after removal from the oven, and like zones were bottled and sealed. Other samples were

allowed to remain on the warm pans in the shop for 15, 30, and 60 minutes, after which each series was zoned and sealed in bottles. Analysis was then made for moisture content. The data are shown in Table IV.

It is evident that under the conditions of the experiment the shape of the biscuit has little, if any, effect on the changes in moisture distribution of the biscuit during the curing process subsequent to baking. The average values shown in the table are shown in graphic form in Figure 4.

It seemed advisable to show the relation between the relative humidity of the air and the moisture content of hard-sweet biscuit in equilibrium therewith. Consequently, hard-sweet biscuit were placed in desiccators over mixtures of sulfuric acid and water until equilibrium was reached, at which time the biscuit failed to change in weight. The moisture contents were then determined. The data are shown in Table V and are graphically illustrated in Figure 5. Each figure is the average of duplicate determinations.

TABLE IV

EFFECT OF SHAPE OF BISCUIT ON RATE OF EQUALIZATION OF
MOISTURE GRADIENT OF FRESHLY BAKED BISCUIT

Type	Moisture content, %			
	Zone 1 (rim)	Zone 2	Zone 3	Zone 4 (center)
Immediately after baking				
Premier	0.93	1.90	2.50	2.80
Algeria	1.03	1.60	2.25	2.68
Arrowroot	0.93	1.50	2.25	2.73
Average	0.96	1.67	2.33	2.74
15 minutes after baking				
Premier	1.13	1.80	2.65	3.10
Algeria	1.40	1.65	2.05	2.35
Arrowroot	1.25	1.60	1.95	2.10
Average	1.26	1.68	2.22	2.52
30 minutes after baking				
Premier	1.35	1.65	2.28	2.70
Algeria	1.25	1.48	1.85	2.15
Arrowroot	1.60	1.85	2.25	2.70
Average	1.40	1.69	2.18	2.52
60 minutes after baking				
Premier	1.50	1.55	2.05	2.45
Algeria	1.60	2.00	2.45	2.60
Arrowroot	1.60	1.85	2.25	2.50
Average	1.57	1.80	2.25	2.52

TABLE V
RELATION OF MOISTURE CONTENT OF HARD-SWEET BISCUIT TO
RELATIVE HUMIDITY OF ATMOSPHERE

Relative humidity, %	Moisture content, %
1	1.05
3	2.05
13	3.45
22	4.36
32	5.70
38	6.43
55	9.10
71	12.48
84	16.50

It seems evident from the results of this experiment, that commercial biscuit vary in moisture content with relative humidity of the atmosphere to which they are exposed. As shown by Wilson and Fuwa (1922), most common foodstuffs and textiles are hygroscopic. This phenomenon being widespread in nature, it seemed logical to assume that biscuit are also hygroscopic. As no figures were available to illustrate this point, it seemed advisable to demonstrate it.
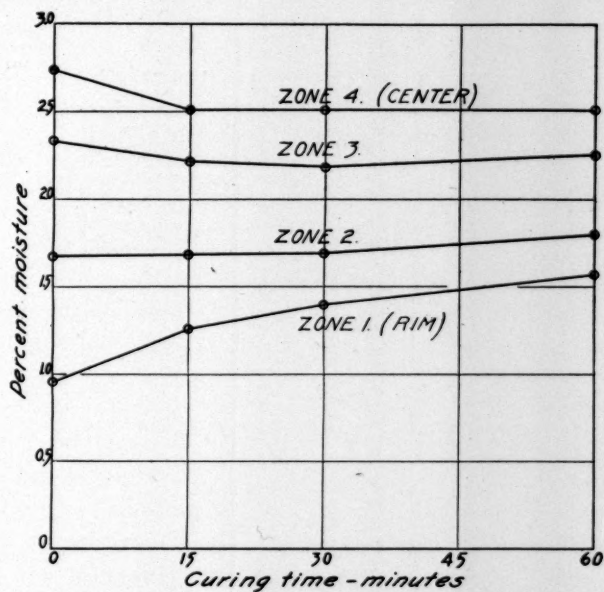
Fig. 4.   Rate of Change in Moisture Content in Different Zones of Baked Biscuit

Another series of experiments was undertaken to show the effect of baking time on the moisture gradient in freshly baked biscuit. The arrowroot biscuit was chosen for this experiment. Samples were baked for 5, 6, 7, and 8 minutes at 224° C. A normal

baking was effected at about 7 minutes, the 5-minute samples being much under-baked, the 8-minute samples over-baked. Immediately after baking, the cookies were zoned, and the zones were subsequently analyzed for moisture content. The data in Table VI are averages of duplicate experiments. Results are shown graphically in Figure 6. A thoro baking appears to decrease the spread in moisture of freshly baked biscuit.

TABLE VI
EFFECT OF BAKING TIME ON MOISTURE GRADIENT OF HARD-SWEET BISCUIT

Time of bake, min.	Moisture content, %			
	Zone 1 (rim)	Zone 2	Zone 3	Zone 4 (center)
5	2.41	5.22	6.01	6.52
6	1.28	2.92	3.53	4.06
7	0.93	1.66	2.33	2.73
8	0.78	1.21	1.60	1.86
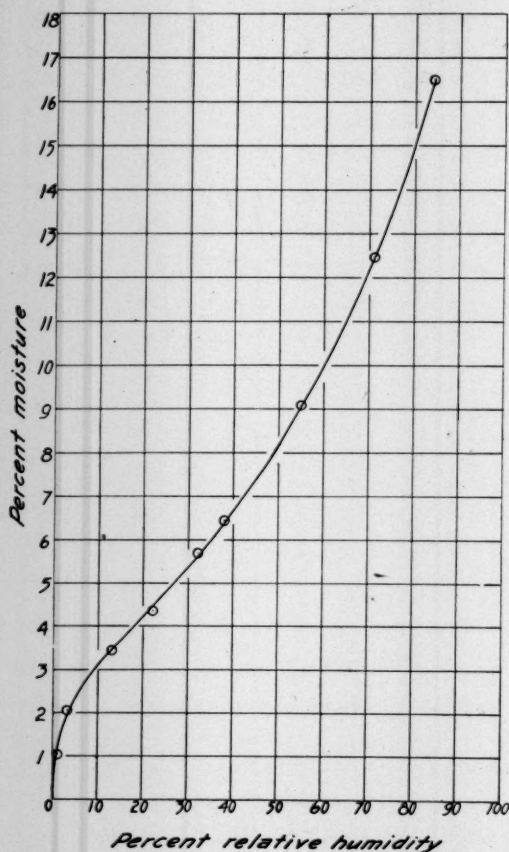
Fig. 5. Relation of Relative Humidity of the Atmosphere to Moisture Content of Hard-Sweet Biscuit

In formulating our hypothesis as to the cause of checking, we assumed that when portions of the biscuit increase in moisture content, they tend to swell; and that when the moisture content decreases, shrinking results. An attempt was made to demonstrate w h e t h e r the changes in size were sufficient to be measured. The method developed was as follows: Biscuit were baked for various lengths of time at 215° C., on a specially constructed wire pan. This pan consisted of a frame a c r o s s which fine steel wires were stretched, forming the support for the biscuit. The wires were 5 millimeters apart and were

all parallel. The resulting biscuit were ribbed on the lower surface with parallel markings. The freshly baked biscuit were mounted up-side-down on a microscope slide, with sealing wax. Care was taken to attach the biscuit to the slide in only one place so that the rest would be free to move or change in dimensions. The distances between markings were measured with a microscope fitted with a mechanical stage and a cross-hair eye-piece. It was thus possible to read dimensions to one-tenth millimeter. All biscuit were measured immediately after baking, and were then placed in desiccators containing a mixture of sulfuric acid and water. Measurements were made at intervals until the curves indicated that the biscuit were approaching equilibrium in moisture content, as conditioned by relative humidities.
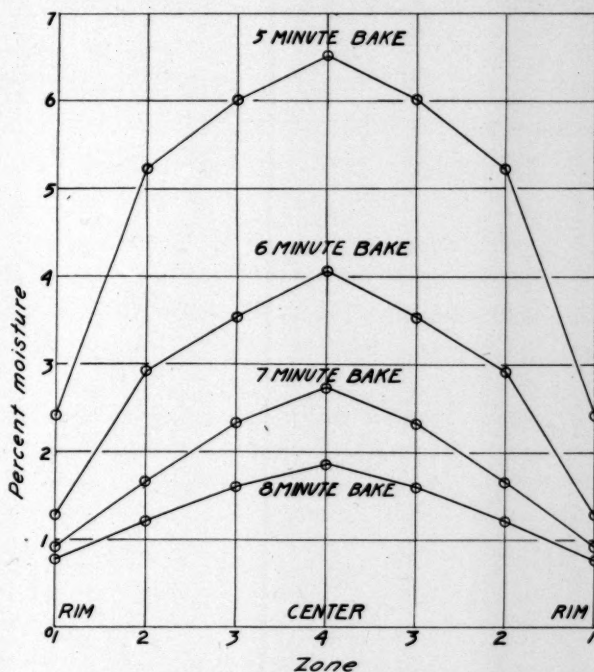
Fig. 6. Effect of Length of Baking Period on Moisture Gradient in Baked Hard-Sweet Biscuit.

This method was sufficiently accurate for measuring the changes in length of the long diameter of the biscuit, but was not accurate enough to measure changes in the central portion. The data are summarized in Table VII and the results are shown graphically in Figure 7.

From the data in Table VII it appears that changes in size of considerable magnitude take place during the curing period,

when the moisture content of the biscuit approaches equilibrium with the relative humidity of the atmosphere. The amount of change seems to depend on the initial moisture content, as conditioned by the time of baking and the relative humidity of the atmosphere.

A more refined method of measuring the changes in dimensions of various portions of biscuit during the process of curing was then devised. Biscuit were baked from hard-sweet dough containing iron filings, which had been carefully cleaned and graded as to size. The filings were of such size that they passed through a
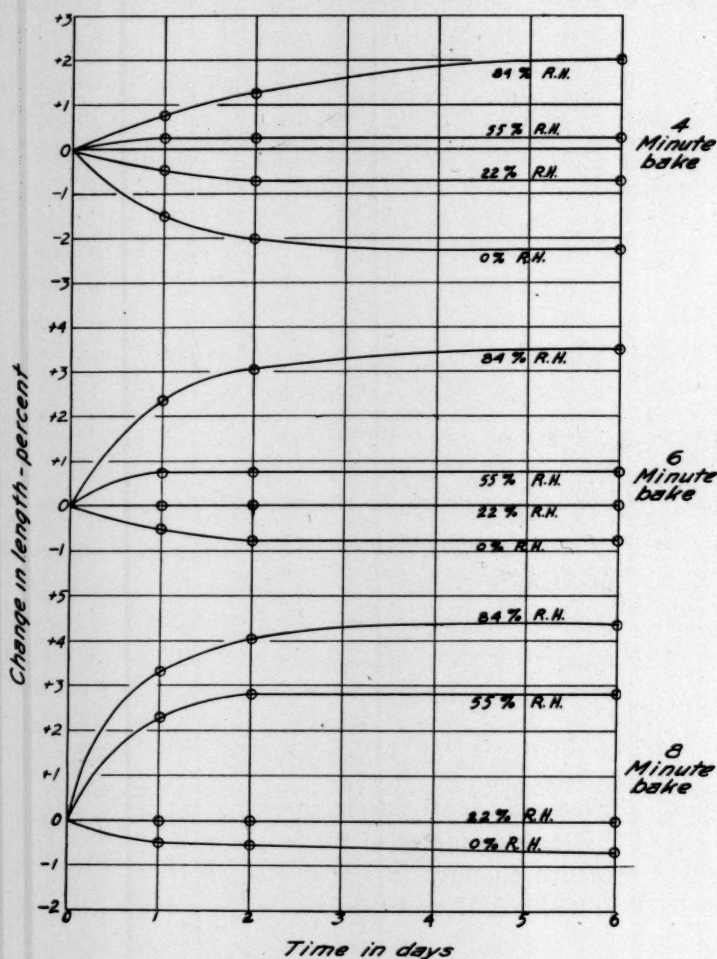
Fig. 7. Relation of Length of Baking Period and Humidity of Curing Chamber to Rate of Change in Moisture Content of Hard-Sweet Biscuit

TABLE VII

Effect of Baking Time and Relative Humidity of Atmosphere During Curing Period on Change in Length of Freshly Baked Hard-Sweet Biscuit

Time of bake, min.	Relative humidity, %	Change in length, %		
		After 1 day	After 2 days	After 6 days
4	0	−1.5	−2.0	−2.2
	22	−0.5	−0.7	−0.7
	55	+0.25	+0.25	+0.25
	84	+0.8	+1.3	+2.0
6	0	−0.5	−0.8	−0.8
	22	0	0	0
	55	+0.8	+0.8	+0.8
	84	+2.3	+3.1	+3.5
8	0	−0.5	−0.55	−0.7
	22	0	0	0
	55	+2.3	+2.8	+2.8
	84	+3.3	+4.1	+4.4

40- and over a 60-mesh bronze screen. The biscuit were baked for 5, 6, and 7 minutes at 218°C. In this case, the 7-minute bake was thoro; the 6-minute bake, slightly underdone, and the 5-minute bake very much underdone. Immediately after baking, contact photographs were taken, using the X-ray. The biscuit was put on an X-ray photographic film, and placed beneath the X-ray equipment. A reference disc of invar, which has a very small index of thermal expansion, was placed on the film as each exposure was made. A positive print from a typical X-ray contact negative may be seen in Figure 8.

After each exposure was made, the biscuit were placed in desiccators held at various relative humidities, and were allowed to come to equilibrium, as indicated by constant weight. Contact photographs were then made as outlined above.

The negatives were mounted between two glass plates, forming an improvised lantern slide; the image was projected on a screen in a dark-room. A special air-
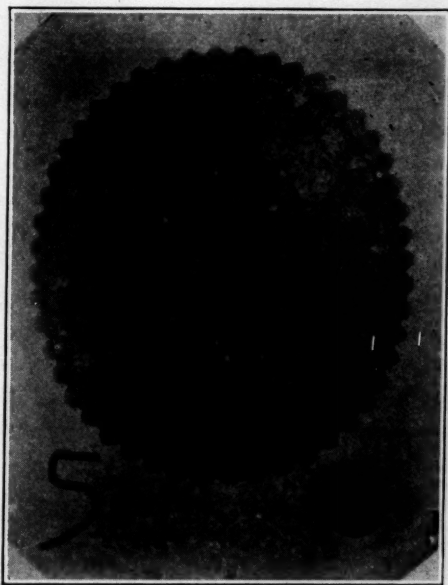

Fig. 8. Positive Print from Typical X-Ray Contact Negative Showing Iron Particles Used in Measuring Swelling and Shrinking in Different Zones of Baked Biscuit.

cooling device was used to keep the negative at approximately the same temperature while measurements were made on the screen. The distances between various "stars" or "constellations" were then measured. In each case, the apparent diameter of the reference disc was measured. As the real diameter of this disc was known with a high degree of accuracy, it was possible to convert the observations on the screen into terms of the distance between points measured on the biscuit. Over 500 such measurements were made. The data were then condensed and typical readings are shown in Table VIII. The data given include changes in the largest diameter of the biscuit and in the central portion, which approximately includes zone 4 (see Fig. 3). The values are expressed as the percentage of change in size, which changes were conditioned by the time of baking and the relative humidity of the atmosphere in which the biscuit were cured.

TABLE VIII

CHANGES IN SIZE OF CENTRAL AND EXTERIOR PORTIONS OF A HARD-SWEET BISCUIT WITH
RESPECT TO BAKING TIME AND RELATIVE HUMIDITY OF CURING CHAMBER

Time of bake, min.	Relative humidity, %	Change in size, %	
		Rim	Center
5	0	−1.05	−1.95
	20	+0.58	−0.49
	55	+0.93	+0.43
	80	+2.31	+1.53
6	0	−0.57	−1.21
	20	+0.39	−0.43
	55	+1.62	+0.69
	80	+3.21	+1.92
7	0	−0.20	−1.15
	20	+0.22	+0.16
	55	+2.38	+1.74
	80	+3.75	+2.98

From the data in Table VIII it appears that when the relative humidity of the atmosphere is about 20% and the biscuit is not thoroly baked, the center shrinks while the outer portion expands. It therefore is natural to assume that a relative humidity of 20% or thereabouts, together with insufficient baking, should cause the biscuit to check if the strain developed in consequence of changes in size exceeds the tensile strength of the portion under strain.

It seemed advisable to determine experimentally the extent to which starch is gelatinized during the process of baking a hard-sweet biscuit. The method used was somewhat similar to that devised by Katz (1915). Hard-sweet biscuit were baked for various

times at a temperature of 218°C. Biscuit were selected that contained the same amount of dry material (5.5 grams) and each was allowed to soak in 50 cc. of distilled water until thoroly soft. Each sample was then rubbed through a 40-mesh bronze screen and the resulting precipitate was washed into a beaker with distilled water. After the precipitate had settled, the supernatant liquid was decanted and the precipitate was washed into a series of 25-cc. graduated cylinders. After settling for one hour, the volume of precipitate was noted. The procedure was carried out in triplicate. The average of the three readings in each case is shown in Table IX, and the results are graphically expressed in Figure 9.
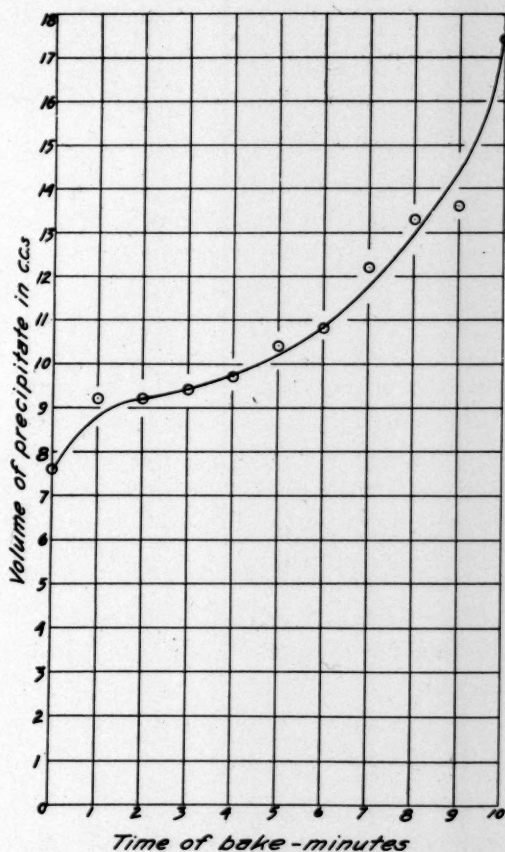
Fig. 9.   Effect of Length of Baking Period on Swelling of Hard-Sweet Biscuit Measured in Terms of Volume of Swollen Crumb

TABLE IX
EFFECT OF BAKING TIME ON SWELLING OF HARD-SWEET BISCUIT CRUMB*

Time of bake, min.	Volume of ppt., cc.	Time of bake, min.	Volume of ppt., cc.
1	9.2	6	10.8
2	9.2	7	12.2
3	9.4	8	13.3
4	9.7	9	13.6
5	10.4	10	17.4

* Volume of unbaked dough, 7.6 cc.

The data in Table IX show that the swelling power of the crumb of hard-sweet biscuit increases during the process of baking. Alsberg and Griffing (1927) have shown that gluten decreases in swelling power when exposed to biscuit-baking temperatures. They conclude that mild heat increases the swelling power of gluten. The maximum swelling power is reached at a temperature between 30° and 50°C. When the temperature is increased beyond this range, the swelling power falls off rapidly. During the first stage of the baking process the swelling power of the biscuit crumb increases rapidly, as both gluten and starch are increasing in swelling power at the same time. In the next few minutes the increase is not so rapid, for the gluten is decreasing in swelling power while the starch is increasing. As the biscuit become thoroly baked (6-7 minutes) the swelling power increases rapidly. It appears, therefore, that considerable gelatinization of starch takes place before the biscuit are removed from the oven.

The fact that the starch, which makes up a considerable portion of a biscuit, is gelatinized during baking is important, as the tendency of the dry portions of the freshly baked biscuit to take up moisture from the moist portions as well as from the atmosphere is thereby increased. Biscuit that are considerably under-baked might be expected not to check to so great an extent as those that have been baked more thoroly, as the gelatinization of the starch has not proceeded so far as in the well baked product. We have previously indicated that thoroly baked biscuit expand more to reach the equilibrium point as conditioned by the relative humidity, than those less thoroly baked.

Discussion

In setting up a hypothesis as to the causes of checking, several assumptions were made. These have been subjected to experimental investigation in order to show their validity. We have

demonstrated that there is an appreciable spread in moisture in the freshly baked hard-sweet biscuit that may be controlled by the conditions of baking. It has also been demonstrated that the various portions of the freshly baked biscuit either gain or lose moisture until all parts of the piece are in equilibrium with the relative humidity of the atmosphere in which the biscuit are cured. It appears that a large proportion of such changes in moisture content take place comparatively soon after the biscuit leave the oven. Further, we have demonstrated that changes in size due to gain or loss of moisture of various portions of the biscuit actually take place, and that these changes are of such magnitude that they may be easily measured. Experimental evidence has been offered which, when viewed in conjunction with some work of Alsberg and Griffing (1927), leads to the conclusion that the imbibitional properties of the starch increase rapidly during the latter part of the baking period.

We have suggested that a highly concentrated sugar solution is present throughout the structure of the biscuit. The suggestion has been made that much of the hardness and rigidity of the biscuit is due to this sugar solution, which is not unlike a stiff taffy or other hard sugar candy. If this is true, a biscuit should be fairly plastic at elevated temperatures, assuming its familiar rigidity as the temperature falls. This conception may be demonstrated by placing biscuit that have been thoroly baked and are quite rigid in the oven and re-heating them. On removal from the oven, they will be found to be plastic and easily bent around the finger. As a biscuit is more plastic at elevated temperatures, it seems logical to suppose that checking might be prevented by allowing the biscuit to equalize the moisture gradient while still warm. Conversely, biscuit that are suddenly cooled after removal from the oven might be expected to be more likely to check than those that are allowed to cool slowly.

As the various suppositions made in setting up the hypothesis as to the causes of checking have been demonstrated to be valid, the hypothesis becomes a theory. This theory may be stated as follows: When freshly baked biscuit leave the oven there is an appreciable spread in moisture content between the inner, more moist portion and the outer, drier portion. This moisture gradient tends to equalize until all portions of the biscuit are in hygroscopic equilibrium with the relative humidity of the atmosphere of the curing chamber. Most of this equalization takes place fairly soon

after baking. If the biscuit is not so thoroly baked as to reduce the moisture gradient sufficiently, and when the relative humidity of the curing chamber is low (about 20%), the center shrinks and the rim expands. If the biscuit is allowed to cool quickly, so that it becomes rigid before this moisture equalization takes place, with its accompanying volume changes in the various portion of the biscuit, checking should occur. We should not expect a biscuit that is much under-baked to check badly, as its starch content has not undergone the changes in imbibitional capacity that take place in the late stage of baking. It appears logical that checking should occur in the region between the inner, shrinking zone and the outer, expanding zone. As a matter of fact, checking is observed more often in this portion of the biscuit than in any other.

If this theory is correct, it should be possible to set up conditions experimentally to produce checking or suppress it at will. It seemed advisable to test the validity of our theory by a series of experiments in which checking should be produced and prevented in accordance with the theory advanced.

Application of the Theory

A relative humidity of 20% was set up in a chamber equipped with air-conditioning apparatus. Hard-sweet biscuit were baked for various lengths of time at 218°C. Each batch was divided into two portions, one of which was placed in the dry atmosphere and the other kept in the room where the relative humidity was 40%. After 48 hours, the biscuit were examined for signs of checking. The data are shown in Table X.

TABLE X
EFFECT OF RELATIVE HUMIDITY AND BAKING TIME ON CHECKING

Time of baking, min.	Checked after 48 hours	
	Relative humidity 20%	Relative humidity 40%
4	10 (only slightly visible)	0
5	20	0
6	70	0
7	0	0

The data in Table X indicate that biscuit slightly under-baked (in which the moisture gradient has not been reduced sufficiently) check badly when cured in a dry atmosphere, whereas no checking was noted when the relative humidity of the curing chamber was 40%. It is of interest to note that biscuit considerably under-baked (4- and 5-minute samples) showed less tendency to check than those sufficiently heated to gelatinize a considerable portion of

the starch, thereby increasing the imbibitional properties of the biscuit matrix. This experiment was repeated several times with similar results.

Another series of experiments was conducted to see the effect of warm curing when the biscuit were subsequently placed in a dry atmosphere. On removal from the oven, half the batch was placed in a box in order to keep the biscuit warm as long as possible and to keep the humidity high (moisture given off from the warm biscuit was retained in the curing box). After curing thus for 30 minutes, the biscuit were placed in the dry-room (20% relative humidity). The other half of each batch was placed in the dry-room immediately upon removal from the oven. The biscuit were baked for 6 minutes at 218°C. Checking in the biscuit that were not cured but placed in the dry-room immediately after baking, ranged from 30 to 50%. Checking in those cured for 30 minutes before being placed in the dry-room was always less than 10%. The biscuit often failed to show any signs of checking. It therefore appears that keeping the freshly baked biscuit warm and in a humid atmosphere will alleviate checking.

Another series of experiments was undertaken to show the effect of sudden cooling on the amount of checking. Each batch of biscuit was divided into three portions. One portion was allowed to cool normally in an atmosphere of 30% relative humidity. Another portion was placed in an ice-box until thoroly cooled and then allowed to dry in the same room as the first. The third portion was cooled with an electric fan and then placed with the other two portions and allowed to cure. In all these experiments, over 80% of the biscuit that were suddenly cooled, checked. Of those allowed to cure naturally in the room, less than 40% checked in each case. It therefore appears that sudden cooling is likely to increase checking.

In all the foregoing experiments, results have substantiated the theory of checking as already outlined. The theory has also been tested in commercial bakeries. In several instances, biscuit bakers have enclosed the conveyors in which the biscuit are cooled, thereby retarding the process of cooling and keeping the relative humidity high, with very beneficial results. In one plant, operating in a dry region, an air-conditioning unit has been installed, and warm moist air is allowed to circulate about the freshly baked biscuit. By this procedure, checking has been practically eliminated. Biscuit bakers have also substantiated the fact that a

very thoro baking, which reduces the moisture spread in the fresh-
ly baked biscuit, helps materially to eliminate checking.

Investigation of Other Factors that Influence Checking

Checking has annoyed the biscuit baker for many years, and
naturally many theories have been advanced for its control. It
seemed advisable to investigate some of these ideas and to note
their effect.

The first series of investigations was designed to ascertain
whether the protein content of the flour had any effect on checking.
Samples of 11 soft wheat flours, and one sample each of hard winter
wheat flour and of spring wheat flour were made up into hard-
sweet biscuit. All samples were baked for 6 minutes at 230°C. Im-
mediately after removal from the oven the biscuit were placed in
the dry-room, where the relative humidity was 20%. After 24
hours, the samples were examined for evidence of checking. The
results are given in Table XI, and graphically shown in Figure 10.
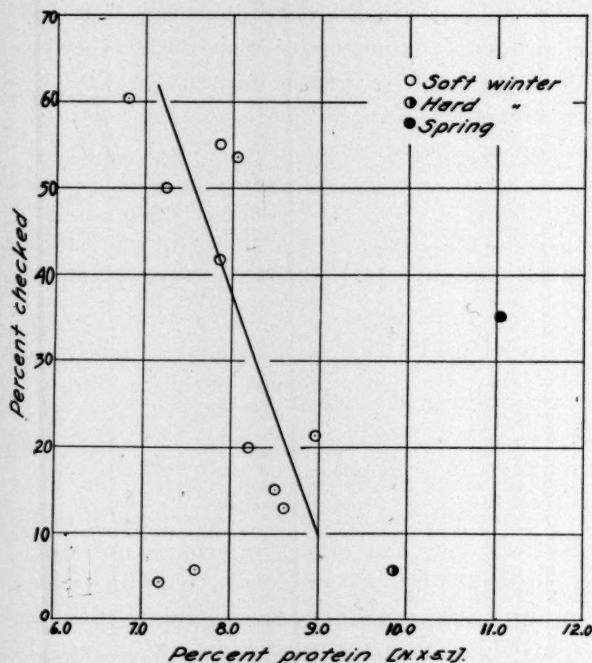
Fig. 10.   Relation of Protein Content of Flour to Relative Checking of
Hard-Sweet Biscuit Baked Therefrom

TABLE XI
RELATION OF PROTEIN CONTENT OF FLOUR TO CHECKING

Source of sample	Protein content, % (N × 5.7)	Checking, %
Michigan	7.6	5.7*
Washington	6.8	60.3
Michigan	7.2	4.3*
Washington	7.25	50.0
Michigan	7.85	41.7
Missouri	7.85	55.0
Oregon	8.05	53.5
Kentucky	8.2	20.0
Illinois	8.5	15.0
Kentucky	8.6	13.0
Missouri	8.95	21.4
Kansas (hard winter)	9.85	5.7
Spring wheat	11.05	35.2

* See discussion of these flours in text.

The data in Table XI indicate that biscuit made from flour too low in protein may check to a greater extent than those made from flour that is higher in protein. That there are other factors entering into the choice of flour for the manufacture of hard-sweet biscuit is indicated by two samples made from Michigan flour, which, altho low in protein, showed little tendency to check. These samples (indicated by * in Table XI) were milled by the same company and were remarkably uniform in granulation. Possibly this evenness of granulation may have some effect on the power of a flour to withstand the forces that cause biscuit to check.

The next factor investigated was the effect of mixing on checking. Six batches of dough were mixed with slow speed until all the flour and the starch had been stirred in. The samples were then mixed for various times with the second speed (see appendix for mixing details) and made into hard-sweet biscuit. All samples were baked for 6 minutes at 230°C. After baking, they were placed in the dry-room at 20% relative humidity, and after 24 hours were examined for traces of checking. The data are shown in Table XII, and graphically expressed in Figure 11.

TABLE XII
EFFECT OF MIXING ON CHECKING

Time of mixing, min.	Checking, %
0	66.6
1	88.8
3	83.3
5	77.8
10	33.3
15	22.2

The data in Table XII indicate that under-mixing is likely to increase checking. It was impossible to continue the mixing longer than 15 minutes, as the doughs were heating so badly that with longer mixing there was danger of losing some of the effect of the leavening agents.
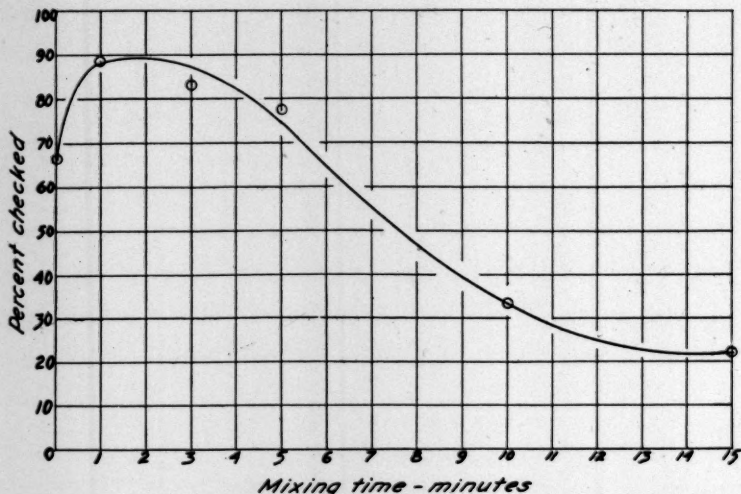

Fig. 11. Effect of Length of Mixing Period on Relative Checking of Hard-Sweet Biscuit

The effect of invert syrup on checking was then investigated. The invert syrup used was made by adding tartaric acid to a boiling hot sugar solution. The syrup was then rapidly cooled to prevent discoloration due to caramelization. Various quantities of invert syrup were added to the standard hard-sweet dough (see Appendix) and biscuit were subsequently baked for 6 minutes at 230°C. The freshly baked biscuit were placed in the dry-room at 20% relative humidity. After 24 hours they were examined for signs of checking. The data in Table XIII show the actual percentage of the total sugar added in the form of invert. The values given take into consideration the fact that inversion was not complete. Compensation for the uninverted sugar in the syrup was made in all cases except that in which 100% of the sugar was to be added in the form of invert sugar. In this case, 96% of the total sugar was invert and the rest was cane sugar. The data in Figure 12 demonstrate that this small discrepancy does not alter the shape of the curve.
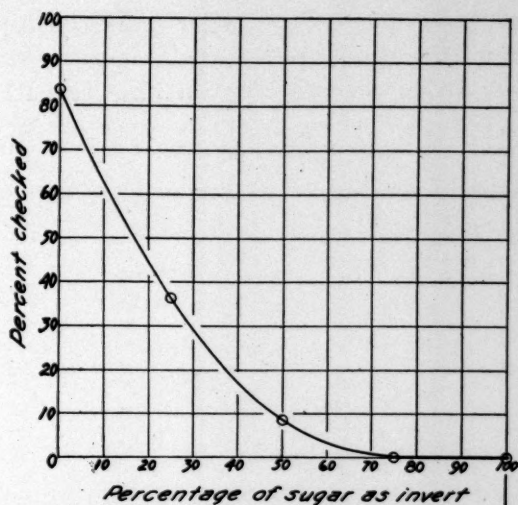
Fig. 12. Relation of Proportion of Sugar Present as Invert
Sugar to Relative Checking of Hard-Sweet Biscuit

The data in Table XIII indicate that invert sugar tends to
eliminate checking. This may be the result of two causes: (1) Just
as candy containing invert sugar is softer than that made from
cane sugar, so biscuits containing invert sugar are softer and more
pliable than those made from cane sugar. (2) Invert sugar tends to
hold more moisture than cane sugar, when the biscuit are in equili-
brium with the relative humidity of the atmosphere. This point
was investigated by analyzing biscuit that had been held for sev-
eral days in an atmosphere containing 20% relative humidity. The
data are given in Table XIV, and expressed graphically in
Figure 13.

TABLE XIII
EFFECT OF INVERT SUGAR ON CHECKING

Total sugar as invert, %	Checking, %
0	83.3
25	36.1
50	8.33
75	0
100	0

TABLE XIV
EFFECT OF INVERT SUGAR ON HYGROSCOPICITY OF HARD-SWEET BISCUIT

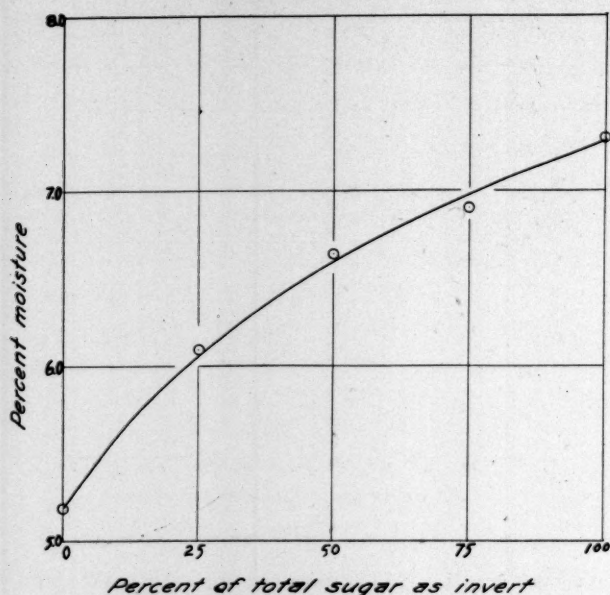| Total sugar as invert, %	Moisture at 20% relative humidity, %
0	5.18
25	6.09
50	6.64
75	6.9
100	7.3

Fig. 13. Relation of Proportion of Total Sugar Present as Invert Sugar
to Hygroscopic Moisture of Hard-Sweet Biscuit

The effect of shortening was investigated by baking samples
containing various amounts of shortening. In all other respects
the standard procedure was followed (see Appendix). After bak-
ing, the samples were placed in the dry-room at 20% relative
humidity. After 48 hours they were examined for checking. The
data are given in Table XV, and shown graphically in Figure
14. As absorbtion varies with the amount of shortening, as already
mentioned, these values are also given.

TABLE XV
EFFECT OF SHORTENING ON CHECKING AND ABSORPTION

Shortening*	Checking, %	Absorption, %*
0	11.1	42.5
7½	41.7	35.0
15	100.0	31.25
22½	8.3	27.5
30	0	21.25
37½	0	15.0

* These values are expressed in the way familiar to the baker, i.e., weight of shorten-
ing or water divided by weight of flour.

The data shown in Table XV indicate that the range in which
checking should prove troublesome is approximately 5-20% of
shortening. This includes hard-sweet biscuit, snaps, and sugar
cookies. This conclusion is further supported by actual experience,
as these types check most.

Other factors investigated were potassium bicarbonate, which might be substituted for sodium bicarbonate, dried skimmilk and whole buttermilk. Data indicate that under the conditions of our experiments these products had little or no effect on the occurrence of checking.
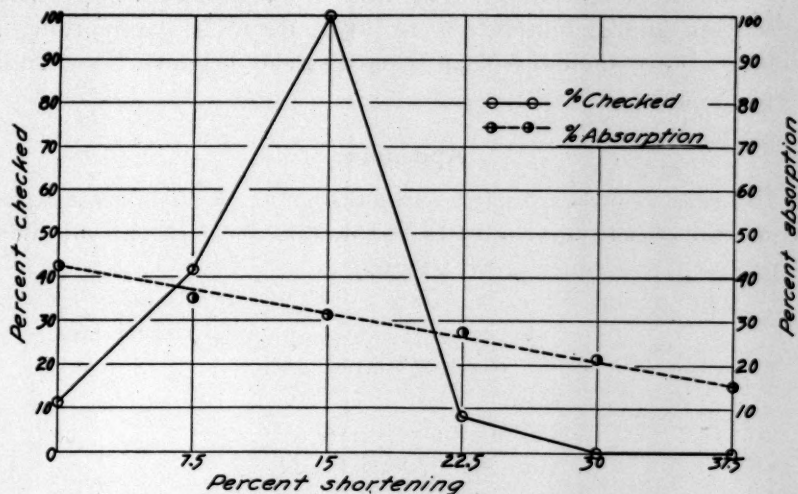

Fig. 14.   Effect of Varying the Proportion of Shortening on the Water Absorption of Dough and Relative Checking of Hard-Sweet Biscuit

Conclusions

1. When a biscuit is removed from the oven there is an appreciable difference in the moisture content of the inner, more moist, portion and the outer, drier, portion. This moisture gradient can be regulated by the baking procedure.

2. During the curing period, this moisture gradient tends to diminish. All portions of the biscuit tend to come into hygroscopic equilibrium with the relative humidity of the curing chamber.

3. The portions of the biscuit that absorb moisture in order to reach this equilibrium increase in size, or swell. Those that lose moisture, shrink.

4. When the relative humidity is low (20%) and the biscuit is not thoroly baked, the center shrinks and the rim expands. If this change in size goes on after the biscuit has become rigid, checking will occur whenever the strain developed exceeds the elastic limit of the section under strain.

5. Conditions that will prevent checking are (in order of their importance) : thoro baking, curing in a humid atmosphere, keeping

the biscuit warm as long as possible, thoro mixing, and inclusion of some invert sugar in the formula.

6. During baking, the swelling power of a hard-sweet biscuit increases, particularly during the last stages. This is probably due to the gelatinization of the starch.

7. Biscuit containing sugar and whose shortening content varies from approximately 5% to 20%, are most susceptible to checking. This includes snaps, hard-sweet biscuits, and sugar cookies.

Appendix

In order to produce hard-sweet biscuit on the laboratory scale that would closely approximate commercial biscuit, the following formula and procedure were adopted:

400 grams Flour
160 " Cane sugar (unless specified otherwise)
60 " Fat (hydrogenated cottonseed oil used)
2 " Salt
2 " Sodium bicarbonate
1 " Ammonium bicarbonate
20 " Arrowroot starch
Water to proper absorption

The fat and the sugar were creamed, using the first speed, then the second speed, on the mixing machine. The ammonia was dissolved in about 30 cc. of water and added to the creamed sugar and fat. Then the salt was added. The creaming was continued until the mixture was light and fluffy. Then the flour (to which the soda had already been added) was stirred in with the first speed. After the mixture was well stirred together, the starch was added. Once the starch had been mixed in, the machine was put in second-gear and mixing was continued for 3 minutes unless otherwise specified.

After mixing, the dough was passed between rolls that were set 3/16 inch apart. The top of the dough sheet was lightly dusted with flour, the sheet was folded and passed through the rolls again. This process was repeated 10 times. Then the dough was rolled up and left for 60 minutes. At the end of this period, it was unrolled and passed thro the break 5 times as before. The rolls were then set about 1/16 inch apart and the dough was rolled through. Biscuit were cut from the dough sheet with the cutter illustrated in Figure 2.

Baking was done in the oven shown in Figure 15, which was fitted with a piece of transite to keep the heat in when the door was opened. The pan was put in through a small opening in the transite. The temperature of baking was read on a thermometer inserted through the top of the oven, the bulb of which was about half an inch above the center of the pan of biscuit.
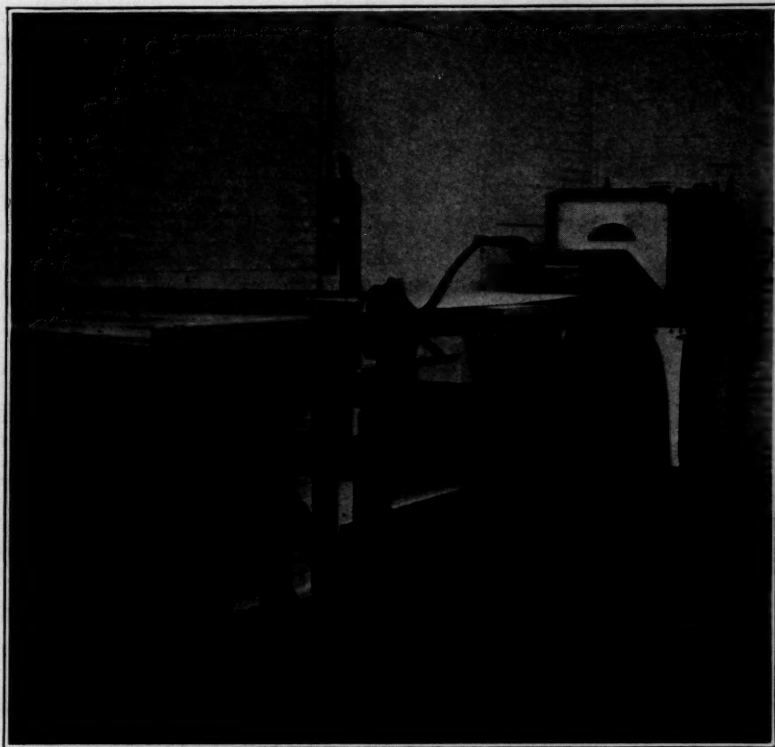

Fig. 15. Special Experimental Gas-Heated Oven and Hand-Operated Rolls Used in Producing Hard-Sweet Biscuit

Literature Cited

Alsberg, C. L.
    1927   Starch in flour. Cereal Chem. 4:485-492.
Alsberg, C. L., and Griffing, E. P.
    1927   The heat coagulation of gluten. Cereal Chem. 4:411-423.
Bingham, E. C. and Jackson, R. F.
    1917   Standard substances for the calibration of viscometers. U. S. Bureau of Standards. Scientific paper 298.
Harkins, W. D., Brown, F. E., and Davies, E. C. H.
    1917   The structure of the surfaces of liquids, and solubility as related to the work done by the attraction of two liquid surfaces as they approach each other. J. Am. Chem. Soc. 39:354-364.

Harkins, W. D., Davies, E. C. H., and Clark, C. L.
    1917   The orientation of molecules in the surfaces of liquids, the energy relations at the surfaces, solubility, adsorption, emulsification, molecular attraction, and the effect of acids and bases on the interfacial tension. J. Am. Chem. Soc. **39**:541-596.

Harkins, W. D., Clark, G. L., and Roberts, L. E.
    1920   The orientation of molecules in surfaces, surface energy, adsorption, and surface catalysis, V. The adhesional work between organic liquids and water. J. Am. Chem. Soc. **42**:700-712.

Harkins, W. D., and Cheng, Y. C.
    1921   The orientation of molecules in surfaces VI. Cohesion, adhesion, tensile strength, tensile energy, negative surface energy, interfacial tension, and molecular attraction. J. Am. Chem. Soc. **43**:35-53.

Harrel, C. G.
    1927   A gravimetric penetrometer for measuring stiffness or toughness of doughs. Cereal Chem. **4**:283-290.

Katz, J. R.
    1915   Das Altbackenwerden der Brotkrume von Physiologisch-chemischen Standpunkte betrachtet. II. Mitteilung. Z. Physiol. Chem. **95**:136-146.

Langmuir, I.
    1917   The constitution and fundamental properties of solids and liquids. II. Liquids. J. Am. Chem. Soc. **39**:1848-1906.

Ostwald, W.
    1919   Beitrage zur Kolloidchemie des Brotes. I. Koll. Z. **25**:26-45.

Platt, W.
    1922   Biscuit and cake manufacture I. Chem. Age. **30**:157-160.

    1922   Biscuit and cake manufacture II. Chem. Age. **30**:203-206.

Platt, W. and Fleming, R. S.
    1923   The action of shortening in the light of the newer theories of surface phenomena. Ind. Eng. Chem. **15**:390.

Sharp, P. F. and Gortner, R. A.
    1923   Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. Minn. Agr. Expt. Sta. Tech. Bull. 19.

Wilson, R. E. and Fuwa, T.
    1922   Humidity equilibria of various common substances. J. Ind. Eng. Chem. **14**:913-918.

SOME OXIDIZING EFFECTS OF FLOUR BLEACHING[1]

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Introduction

During the storage of unbleached flour two obvious changes occur—the destruction of part of the yellow color, and an improvement in baking characteristics. The same types of change occur when flour is chemically bleached, in which case the change in baking characteristics is often known as "maturing." The chemistry of the actual bleaching is probably fairly well understood, and will not be discussed in this paper. On the contrary, the exact chemical changes that cause the improvement in baking quality brought about by ageing or maturing are still but little known. Probably the increase in hydrogen-ion concentration is the only factor in the change which has been explained upon sound scientific grounds.

Experimental Procedure

It has been shown by the author (1928) that the addition of acid and an oxidizing agent will take the place of the fermentation period in developing a dough. Such a "no-dough-time" method constitutes an excellent means of investigating the oxidizing effects of different methods of bleaching. For this study, samples of an 85 per cent patent hard winter wheat flour were taken from a 75-barrel mill, one unbleached, one bleached with $2\frac{1}{4}$ grams, and one with $7\frac{1}{2}$ grams nitrogen trichloride per barrel. Four additional samples of the unbleached flour were treated, respectively, with chlorine containing 0.5 per cent of nitrosyl chloride at the rates of 45 and 67 grams per barrel, and with a commercial preparation of benzoyl peroxide at the rates of 18 and 27 grams per barrel.

These samples were baked the day after milling and again three weeks later by the following formula:

Flour	340 grams
Yeast	10 grams
Sugar	12 grams
Salt	6 grams
Shortening	4 grams
Lactic acid (90%)	8.5 cc.

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[1] Contribution No. 36, Department of Milling Industry.

Mix 1½ minutes at 120 revolutions per minute, scale off 500 grams, and pan immediately. Proof to standard volume (3 cm. rise on the closed cylindrical pan of Swanson and Willard)[2] and bake. At each baking a sample of unbleached flour was baked with the addition of 0.01 gram of sodium chlorate to the dough. Table I shows the loaf volume and texture resulting.

TABLE I

Treatment	Aging	Absorption	Loaf vol.	Texture
		per cent	cc.	per cent
Unbleached	1 day	63	1720	88
Unbleached plus 0.01 g. Na ClO₃	"	63	1940	99
2¼ g. NCl₃	"	65	1870	95
7½ g. NCl₃	"	66	1950	99
45 g. Cl	"	65	1820	90
67 g. Cl	"	65	1925	98
18 g. Benzoyl peroxide preparation	"	65	1840	92
27 g. " " "	"	65	1835	92
Unbleached	22 days	71	1880	94
Unbleached plus 0.01 g. Na ClO₃	"	71	1985	99
2¼ g. NCl₃	"	69	1930	98
7½ g. NCl₃	"	66	1890	94
45 g. Cl	"	67	1900	96
67 g. Cl	"	68	1980	99
18 g. Benzoyl peroxide preparation	"	67	1915	96
27 g. " " "	"	67	1950	99

The unbleached sample and the samples bleached with nitrogen trichloride were also baked after a fermentation period of 2 hours and 25 minutes at 32°C. (90°F.), in which cases the lactic acid was omitted from the dough and the sugar increased to 15 grams. The entire dough, about 600 grams, was used. The results are shown in Table II.

TABLE II

Treatment	Aging	Absorption	Loaf vol.	Texture
		per cent	cc.	per cent
Unbleached	1 day	61	2095	96
2¼ g. NCl₃	"	63	2125	97
7½ g. NCl₃	"	64	2040	95
Unbleached	22 days	69	2145	97
2¼ g. NCl₃	"	67	2130	97
7½ g. NCl₃	"	64	1990	94

Results

The bleaching of flour is seen to have the same or a similar effect in developing the dough made from that flour as the addition of oxidizing agents to the dough. This effect is seen to persist after three weeks standing, except that in the case of the treat-

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[2] Kansas Agr. Expt. Sta. Bull. 177. p. 92.

ment with 7½ grams of nitrogen trichloride the action seemed to have been excessive. With benzoyl peroxide in the amounts used, and with the other bleaching agents in the small amounts, the results after three weeks were better than those immediately after treating. The addition of an oxidizing agent such as sodium chlorate to the dough does not have a bleaching effect.

The fact that the absorption of the unbleached flour was notably higher than that of the bleached after standing only three weeks was a surprise, and moisture determinations showed that the moisture content of the different flours was the same within less than 0.1 per cent. This seems to indicate that, at least in this case, the bleached flour was in some respects already beginning to deteriorate, in comparison with the unbleached.

Discussion

The attempt has often been made to explain the improvement in baking quality of flour due to aging and to maturing as being the result of the increase in H-ion concentration. That this explanation is not sufficient may be seen from several facts. The addition of acid alone to the dough will not entirely take the place of aging or maturing. Bleaching methods using nitrogen trichloride or benzoyl peroxide have very little effect upon H-ion concentration, but are very effective in maturing the flour. (In the case of benzoyl peroxide the maturing effect is slow).

All living cells contain certain fat-like substances called phosphatides. These phosphatides are to some extent soluble or capable of being dispersed in water, are very unstable, and are believed to be instrumental in the process of respiration. Any substances as unstable as the phosphatides are sure to be materially altered by the powerful oxidizing agents such as are used in flour bleaching. The respiration of wheat and flour is probably an important factor in aging, and consequently a change in the phosphatides would be expected to accompany natural aging in flour.

The phosphatides in a flour have been shown to have an important influence on the baking qualities of that flour. As the same changes that may be expected materially to alter the phosphatides have an important effect on the baking qualities of the flour, it is reasonable to look for a causal relationship between the two.

The deleterious effect upon bread dough of the addition of quantities of phosphatide of the order of 0.5 to 1% has been noted by the author. (1924). At that time it was not fully realized

that a smaller amount, such as that normally present in a good patent flour, might possibly have a beneficial effect. The evidence on this point is not yet conclusive, but seems to indicate that the latter is the case. The beneficial effect of small amounts of oxidizing agents seems to be due to their action in rendering phosphatides more soluble in water. Whether the detrimental effect of excessive oxidation might be due to the destruction of phosphatide, to the liberation of too much phosphatide, or to a destructive effect on the protein itself, is as yet unknown. There is also need for intensive study of the requirement of the lower grades of flour for more vigorous oxidation than the patent flours will stand. The higher ash content of these flours might so decrease the dispersion of phosphatide as to require the liberation of a greater amount, or, on the other hand, the larger quantity of phosphatide present might need to be partially destroyed by oxidation.

After milling, the quality of normal flour improves for some time, reaches a maximum, and then begins to deteriorate. Preliminary experiments and general observation indicate that this deterioration begins sooner in bleached flours than in unbleached. Flour cannot be so bleached at the mill as to insure optimum baking quality at the time of baking, as this time cannot be known in advance, and the degree of oxidation required varies with the baking procedure. Consequently, if the baker is to obtain best results, he must in many cases add an additional oxidizing agent. It seems possible that larger bakeries could secure several important advantages by buying unbleached flour and bleaching shortly before use. By this means the period of maximum quality in the flour would be prolonged and the severity of oxidation could be accurately adjusted to the needs of the flour at the time and to the baking procedure used. The use of proprietary materials of unknown composition could also be dispensed with, and yeast foods and salts effective in toughening gluten be added in the most desirable quantities without at the same time using too much or too little of some oxidizing agent.

Summary

The bleaching of flour has developed along such lines that the actual destruction of color is scarcely more important than the accompanying effects generally known as "maturing." Theoretically, this maturing should be similar to the development obtained in dough by the use of oxidizing agents.

The use of lactic acid and a heavily over-bleached flour will give the same results in developing a dough for a no-dough-time baking method as will the use of acid with sodium chlorate or other oxidizing agents.

The quality of normal flour improves for some time after milling, reaches a maximum, and then begins to deteriorate. Preliminary experiments and general observation indicate that this deterioration begins sooner in bleached flours than in unbleached. Thus it seems possible that large bakeries could prolong the period of maximum quality in their flour by buying unbleached flour and bleaching shortly before use. In this way the severity of the oxidation could be accurately adjusted to the needs of the flour at the time and to the fermentation schedule desired.

Literature Cited

Working, E. B.
    1928   The action of phosphatides in bread dough. Cereal Chem. 5: 223.
    1924   Lipoids, a factor influencing gluten quality. Cereal Chem. 1: 153-158.